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THE UNIVERSITY OF ALBERTA

EVALUATION OF N-GLUCOSYL UREIDE PREPARATIONS AS NON-PROTEIN NITROGEN SOURCES FOR RUMINANTS

by

PAUL JEROME MARTIN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

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DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING, 1976

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Evaluation of N-glucosyl Ureide Preparations as Non-protein Nitrogen Sources for Ruminants" submitted by PAUL JEROME MARTIN in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Nutrition.

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ABSTRACT

Experiments were conducted to assess the efficacy of N-glucosyl ureide (GU) and N-glucosyl-N'-hydroxymethyl ureide (MGU) as nitrogen (N) supplements in ruminant diets. The results of a trial in which liquid supplements containing GU or MGU were compared to soybean meal and urea as N sources in diets for growing calves indicated that the use of the liquid supplement containing MGU depressed average daily weight gain while the addition of the other N supplements to the basal diet did not improve average daily gain due, perhaps, to the unexpectedly high rate of gain of the control group. The animals fed the supplements containing GU or MGU did not exhibit any apparent signs of toxicity.

A solid preparation of GU (BBGU), manufactured from barley and urea, was compared to soybean meal as an N supplement in diets for steers. During period 1 of the experiment, diets of low digestible energy (DE) concentration were used; in period 2, diets high in DE were fed. In period 1, animals fed soybean meal gained more rapidly than those in the control group while in period 2, animals fed BBGU gained at a more rapid rate than those fed soybean meal ($P < 0.05$). The use of BBGU or soybean meal, however, did increase dry matter (DM) intake and plasma

urea nitrogen (PUN) concentrations but had no effects on fat cover or loin eye area of the carcasses.

A digestibility and N balance study was undertaken to determine the DE concentrations of BBGU and BBMGU, a preparation of MGU manufactured from barley, urea and formaldehyde. The estimated coefficient of N digestibility of BBGU (105.5%) was higher than that of BBMGU (75.6%). There were no differences between BBGU and BBMGU with respect to apparent DM or energy digestibility. Estimated DE values (cal/g) for BBGU and BBMGU were 3488 and 3002, respectively.

The effects of BBGU, BBMGU, urea and soybean meal on rates of ammonia accumulation in the rumen were determined in an experiment in which fistulated steers were fed the N supplements over a 15-day period, with the supplements being administered intraruminally on days 1, 8 and 15. The administration of urea (30 g of N) increased ruminal ammonia concentrations more rapidly than the administration of BBGU, BBMGU or soybean meal, each of which was added in an amount sufficient to supply 60 g of N. There was apparent adaptation to BBGU during the first seven days of feeding.

An experiment in which lambs were fed, *ad libitum*, concentrate mixtures containing 10% soybean meal, 10%, 20%,

50% and 99.85% BBGU (plus 250 g of alfalfa per head per day) for 63 days was used to determine the potential toxicity of BBGU. Lambs fed the concentrate mixture containing 99.85% BBGU lost weight at an average rate of -0.03 kg per day during the trial. There were no significant differences in rate of gain among the other treatment groups. *Post mortem* examination of the brains of sheep in the groups fed 50% or 99.85% BBGU showed areas of demyelination, axon swelling and degeneration.

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INTRODUCTION

The use of grains and protein supplements as animal feeds, in view of present world food shortages, may be considered wasteful since they may be used directly as human food. The utilization of such products in ruminant diets might be considered to be especially wasteful since ruminants generally require more dietary dry matter per unit of live-weight gain than domestic monogastric animals such as swine or poultry.

Ruminants, however, can utilize dietary cellulose and, thus, can be fed fibrous agricultural and industrial products that otherwise might not be used for food production. Since fibrous plant material, in terms of mass, is the major product of photosynthesis, the future of the ruminant as an important supplier of human food most probably is assured.

Protein is one of the more costly ingredients in animal diets. However, ruminants have the ability to utilize non-protein nitrogen (NPN) in lieu of nitrogen (N) from dietary protein. Thus, urea, which is a simple, inexpensive chemical, has been used to supply dietary N for ruminants. Ingested urea is hydrolyzed to ammonia and carbon dioxide but, unless readily-fermentable carbohydrate

is simultaneously available for use by the rumen microbes for derivation of energy and carbon for synthesis, poor utilization of N will result. However, urea generally has not been as effective as conventional protein sources even when diets with high contents of starch or other readily-fermentable carbohydrates have been used (Chalupa, 1968). Problems of availability, mixing, palatability and potential toxicity have been responsible for limitation of usage of urea by livestock producers.

No inexpensive and effective source of NPN for use in diets with high contents of roughage and low contents of digestible energy has yet become widely available. Work at The University of Alberta and The Research Council of Alberta has resulted in the development of two NPN products, N-glucosyl ureide (BBGU) and N-hydroxymethyl-N'-glucosyl ureide (BBMGU) manufactured from barley and urea, both of which appear to yield ammonia in the rumen at rates less than that of urea. Therefore, these products may be superior to urea as sources of dietary NPN and should be examined in experiments in which their effects upon the performance of animals may be measured.

REVIEW OF LITERATURE

Nitrogen metabolism in the rumen

Protein which is ingested by the ruminant may be degraded by microorganisms in the rumen or may pass through the rumen and then be digested in the lower gut or excreted in the feces. The extent to which proteins are degraded in the rumen is at least partially dependent on their solubilities (Tillman and Sidhu, 1969; el-Shazly, 1958). The digestion of protein in the rumen yields peptides and amino acids which may be used directly by the microorganisms, or the amino acids may be acted on by deaminases and deamidases to form ammonia and volatile fatty acids (Hungate, 1966; Hobson, 1969; Tillman and Sidhu, 1969).

Ammonia is utilized by rumen bacteria as a source of nitrogen (N); it is essential for some species and is used in preference to amino acids by others (Hungate, 1966). Milligan (1970) suggested that the syntheses of glutamate from α-ketoglutarate and glutamine from glutamate may be major sites of ammonia fixation in rumen microorganisms. Rumen epithelium apparently is capable of fixing ammonia by means of the formation of glutamine from glutamate (Hoshino *et al.*, 1966; McLaren *et al.*, 1962). Mathison and

Milligan (1971) suggested that fixation of ammonia in the rumen epithelium would tend to protect the animal against ammonia toxicity and reduce the recycling of urea.

Ammonia may be absorbed from the rumen and other parts of the gastrointestinal tract into the portal blood system. The liver converts the absorbed ammonia to urea which is then circulated in peripheral blood. This endogenous urea may enter the rumen via the saliva (Lewis, 1961) or by diffusion (Houpt, 1959). Alternately, the urea may be excreted in the urine.

The hydrolysis of urea to form ammonia and carbon dioxide is catalyzed by bacterial urease (Huhtanen and Gall, 1955). Nitrogen from other sources of NPN also yields ammonia in the rumen (Belasco, 1954). The utilization of ammonia by rumen microorganisms requires carbon skeletons, usually supplied by or synthesized from the intermediates of carbohydrate fermentation and the end-products of fermentation (Chalupa, 1972).

History of the use of non-protein nitrogen as a protein substitute

Weiske (1879) first discovered that asparagine could be utilized by sheep as a source of dietary N. Other European workers (Zuntz, 1891; Hagemann, 1891; Müller, 1906) confirmed the results of Weiske and suggested that

rumen microorganisms were responsible for the utilization of NPN. Subsequently, Honcamp and Koudela (1927) stated that ammonium salts or urea may be used as substitutes for supplemental protein in maintenance and production diets for sheep and cows, if the basal diets were low in protein and rich in carbohydrates. Starch, they suggested, was superior to other carbohydrates in promoting the synthesis of protein from NPN. Hart *et al.* (1938) investigated the use of urea and ammonium chloride by calves. They concluded that NPN could be utilized by ruminants, by means of "bacterial intervention". In Britain, Bartlett and Cotton (1939) found that urea gave a definite growth response in heifers fed a diet with a low content of protein. Virtanen (1966) reported that the synthesis of microbial protein from urea and ammonium salts, provided as the sole sources of N in purified diets, was sufficient to allow production of as much as 4217 kg of milk annually by dairy cows. Virtanen stated that an adaptation period was required to maximize the utilization of NPN in the diet.

Historical sources of non-protein nitrogen

Reid (1953), in a review article, concluded that urea could be used satisfactorily in maintenance and fattening diets at the rate of 25-33% of the dietary N. He stated that, in the case of diets for growing calves, urea

was somewhat inferior to conventional protein supplements.

In 1940, the Association of American Feed Control Officials approved the use of urea and specified that not more than 33% of the total N in a ruminant diet should be in the form of NPN (Association of American Feed Control Officials, 1955). The use of urea in animal feeds in the United States has gradually increased since 1940 with current usage estimated at 800,000 tons per year (Oltjen, 1973).

In Canada, urea was first used in cattle feeds in the early 1950's. The maximum allowable percentage of total N in a grain diet which may be supplied by urea has been 33% in Canada. The Canadian feed industry has used an average of 20,000 tons of urea per year during the period 1971 to 1974. Consumption of urea was expected to reach 40,000 tons in 1975 and 80,000 tons in 1985 (letter dated March 10, 1975, from C.L. Stevenson, Plant Products Division, Canada Agriculture).

Numerous other NPN compounds have been evaluated by researchers using *in vitro* and *in vivo* techniques. Belasco (1954) used an *in vitro* procedure entailing incubation with rumen microorganisms to test urea derivatives, amides, amidines and ammonium salts of organic and inorganic acids as possible NPN supplements. Compounds which showed promise in terms of rate of release of ammonia and degree

of cellulose digestion included guanidine, creatine, creatinine, ammonium formate, ammonium succinate and ammonium lactate.

Biuret, a pyrolysis product of urea, has been utilized as an NPN supplement. Feed grade biuret, marketed in the United States under the trade name of "Kedlor", contains 40% N, a minimum of 55% biuret and a maximum of 15% urea. Biuret is converted to ammonia in the rumen at a comparatively slow rate (Hatfield *et al.*, 1959).

"Starea", an intimate mixture of gelatinized starch and urea (Helmer *et al.*, 1970), was developed and tested at Kansas State University. The use of "Starea" as compared to isonitrogenous and isocaloric mixtures of starch and urea apparently has lowered blood and ruminal ammonia concentrations and increased N retention in lambs (Schiehzadeh and Harbers, 1974).

Urea has enjoyed prominent usage in the concentrate portions of ruminant diets. Urea is produced in massive amounts for use in agricultural fertilizers and, therefore, is readily available to the feed manufacturer. Consequently, urea is the major source of supplemental NPN in ruminant diets. Since urea is used to the virtual exclusion of all other NPN sources, the terms NPN and urea are essentially synonymous in common usage.

Factors affecting the effectiveness of non-protein nitrogen in ruminant diets

Intake of dry matter

If a supplement is to be useful, it must be sufficiently palatable or acceptable to allow for normal rates of total feed consumption when included in diets at recommended levels. The use of NPN rather than protein as the sole source of supplemental N in growing and fattening diets often has resulted in voluntary intakes that are less than those observed for diets supplemented with soybean meal or other sources of protein. Lowrey and McCormick (1969) fed steer calves diets that were high in digestible energy and that contained urea at concentrations of 0%, 0.2% and 1.4%. Voluntary feed intakes (9.61, 9.20 and 8.23 kg per head per day, respectively) tended to decrease as the urea content of the diet increased.

Huber and Cook (1969) provided urea orally as part of the concentrate portion of the diet and compared its effect on feed consumption with that of equivalent amounts of urea placed in the rumen or abomasum. Animals fed a concentrate mixture containing 3% urea consumed less feed than animals that were fed a concentrate mixture containing 1% urea and had amounts of urea equivalent to 2% of the concentrate mixture added to the rumen or abomasum. They concluded that the intake depression observed

was due to the taste of urea.

Van Horn *et al.* (1967) found that the inclusion of 1% urea in a concentrate mixture fed to dairy cows did not cause a decrease in consumption of the mixture. The inclusion of 1.9% urea in the mixture, however, did reduce the amount of concentrate consumed. Young *et al.* (1973) and Schmidt *et al.* (1973) fed steers diets containing 1.2% and 0.9% urea, respectively, and found that the inclusion of urea at these levels did not depress voluntary feed intake.

Digestibility of energy in the diet

Ammonia produced from dietary NPN may be utilized by rumen microorganisms to synthesize amino acids. If ammonia in the rumen is to be utilized for formation of amino acids, carbon skeletons, derived from the fermentation of carbohydrates, must be present when the ammonia is available.

Starch has been considered an ideal source of carbon skeletons in diets containing urea since the rate at which it is fermented seemingly is compatible with the rate at which ammonia is released from urea. Sugars are suggested as being inferior to starch due to the rapid rate at which they are fermented (Schwartz and Shoemann, 1964). Cellulose is inferior to both starch and sugars due to its

low rate of fermentation (Helmer and Bartley, 1971; Oltjen, 1973).

Houpt (1959) fed mature sheep either a basal diet consisting of timothy hay or a diet of timothy hay plus a carbohydrate supplement containing cornstarch and sucrose. He injected urea intravenously and then measured the amount of urea excreted in the urine of the sheep. Houpt estimated that, when only the timothy hay was fed, 22% of the urea was not recovered and, therefore, probably was utilized in the synthesis of protein in the rumen. When timothy hay plus the carbohydrate supplement were fed the proportion of urea retained increased to 52% of the standard dose. McLaren *et al.* (1965) noted increased retention of N from dietary NPN with increased concentrations of readily available carbohydrates (cornstarch, dextrose and cane molasses) in purified diets fed to lambs.

Perry *et al.* (1967) compared supplements in which the majority of the N was supplied by urea with a protein supplement consisting primarily of soybean meal and dehydrated alfalfa meal. In four of the five trials in which diets of high digestible energy contents were used, steers fed the urea supplements gained as rapidly as those fed the natural protein supplement. In the fifth trial, the average daily gain of the animals fed the soybean plus dehydrated alfalfa meal supplement was significantly higher

than those of the NPN groups. When the same supplements were compared using diets of low digestible energy content for growing calves, there were significant differences in growth rates and feed:gain ratios. The natural protein supplement was obviously superior to the urea supplements when diets low in digestible energy were used.

The literature is replete with conclusions very similar to those offered by Perry *et al.* (1967): growth rates and feed:gain ratios obtained with urea and natural protein supplements are similar when they are used in diets that are high in digestible energy (Braman *et al.*, 1973; Lowrey and McCormick, 1969; Oltjen *et al.*, 1965; Haskins *et al.*, 1967); growth rates and feed efficiency ratios obtained with natural protein supplements are superior to those obtained with urea supplements when they are used in diets that are low in digestible energy (Van Slyke *et al.*, 1971; Winter, 1973; Freitag *et al.*, 1968; Lassiter *et al.*, 1958).

Some researchers, however, have reported that natural protein supplements were superior to urea in diets that were high in digestible energy. Schmidt *et al.* (1973) fed steers (average initial body weight of 374 kg) diets containing high concentrations of digestible energy for 56 days. The average daily gain of the group fed soybean meal was significantly higher than that of the group fed urea (1.64 kg vs. 1.36 kg). Young *et al.* (1973) stated

that substitution of a urea-corn mixture for soybean meal in a diet consisting largely of ear corn decreased the average daily gain of yearling steers by 11% and lowered the concentration of plasma free amino acids ($P < 0.05$). Young *et al.* (1973) concluded that the inclusion of soybean meal in the diet resulted in the absorption from the gut of a "quantitative and qualitative pattern of amino acids" that was more "desirable" than that resulting from the inclusion of the urea-corn mixture.

The rate at which ammonia is produced in the rumen

Urea is hydrolyzed rapidly to ammonia and carbon dioxide in the rumen in a reaction catalyzed by microbial urease. The rapid hydrolysis of urea results in the accumulation of ammonia in ruminal fluid. Ammonia may be utilized by rumen microorganisms or, if produced at a rate in excess of the rate at which it is used by the microbes, may be absorbed into portal blood through the walls of the rumen, the omasum and the lower parts of the gut. The ammonia then is transported to the liver and converted to urea which may be excreted in the urine. Some of the urea, however, may be returned to the rumen by diffusion from the blood or via the saliva (Chalupa, 1968).

The fermentation of natural feedstuffs, particularly roughages, occurs over a prolonged period of time.

Hungate (1966) describes a model that predicts the extent of digestion in the rumen with time, based on *in vitro* fermentations and continuous fermentation theory. He states that soluble carbohydrates are utilized at a linear rate over a period not exceeding 1 h and that a second fermentation period, lasting up to 6 h, may represent the digestion of soluble polymer components (e.g. starch, xylans and pectins) and the beginning of utilization of fibre materials. The final period of digestion, which is non-linear with respect to time, is the period in which the more resistant fractions, consisting of "cylindrical fibres containing long polymers arranged in concentric rings", are fermented. Hungate suggests that digestion is essentially complete in 36 h. Thus, in order to maximize N utilization, ammonia should be produced in the rumen over a prolonged period of time, particularly where diets comprised largely of roughages are used.

Bloomfield (1960) found that urea could be hydrolyzed at the rate of 80 mg of urea N per hour per 100 ml of rumen fluid, while ammonia was utilized at the rate of 20 mg of ammonia N per hour per 100 ml of rumen fluid. Hembry *et al.* (1975) reported a definite negative relationship between the rate of ammonia production in the rumen and the utilization of supplemental N fed to sheep.

Huston *et al.* (1974) evaluated an NPN supplement (a mixture of urea, starch and a carboxy resin) by comparing it with urea and cottonseed meal in diets for lambs. *In vitro* trials with rumen fluid suggested that ammonia was produced from the mixture at a slow, sustained rate. Lambs that received the NPN supplement by rumen cannulae had lower blood urea concentrations than those that received comparable amounts of urea in their diets. Lambs fed a growing diet including the NPN mixture gained as rapidly as those fed supplemental cottonseed meal and more rapidly than those fed urea.

Knight and Owens (1973) simulated the action of NPN supplements that produce ammonia at a slow rate in the rumen. A pump was used to infuse urea intraruminally into sheep fed diets of 60%, 65% and 75% dry matter digestibility (DMD). Urea was infused in amounts adequate to increase the equivalent protein concentration of the diets to 12%. Urea was infused during the first hour after feeding, during the first 3 h after feeding or for 12 h after feeding. A fourth treatment group into which urea was not infused also was included in the trial. Three lambs were assigned to each treatment. A 9-day adjustment period was followed by a 5-day period during which feces and urine were collected.

Sheep fed the 75% DMD diet did not benefit, in

terms of N balance or DMD, from extending the daily period from 1 h to 3 h or 12 h. When the 65% DMD diet was fed, the 3 h infusion period provided slightly greater N retention than the other periods. Results for the 60% DMD diet were similar to those for the 65% DMD diet: sheep receiving urea over the 3 h period had a slightly higher N balance than those receiving urea during the 0, 1 and 12 h intervals. The DMD was not affected by the infusion treatments. The authors suggested, on the basis of these results, that the use of N supplements that produce ammonia at a relatively slow rate in the rumen may be advantageous in diets of 60-65% DMD.

Concentration of nitrogen in the diet

In experiments designed to measure the effectiveness of NPN supplements, a control group fed a diet containing an inadequate concentration of protein should be included to establish the potential in the trial for measurement of response to dietary protein. If such a control were not included and a group of animals provided with NPN exhibited performance equal to that of a group provided with supplemental protein one might conclude validly that the NPN and protein were equally effective, or equally ineffective. Thus, one should be very cautious in accepting the statement of Lichtenwalner *et al.* (1973) that urea,

corn gluten meal and soybean meal were equally effective in a fattening diet when it was not established that there would have been any response to supplementation. Similarly, the conclusion of Lowrey and McCormick (1969) that urea was equivalent to cottonseed meal as an N supplement in diets for steers is suspect for the same reason.

Satter and Roffler (1975) employed an *in vitro* technique using continuous-culture fermentors charged with rumen ingesta to assess the effect of increasing supplies of N, in the form of urea, on the amount of protein produced in the fermentor. They found that protein production reached a plateau at the point where ammonia began to accumulate. When ammonia N levels exceeded 5 mg per 100 ml of rumen fluid (3.6 mM), protein production did not increase. The authors then obtained a total of 1,038 samples of rumen ingesta from 207 lactating cows fed a variety of diets in which only natural protein was used. Mean ruminal ammonia concentrations were calculated from the concentrations in samples taken prior to feeding and at least three times after feeding. A positive correlation was found between mean ruminal ammonia concentration and the level of crude protein in the diet. When the diets contained more than 13% crude protein, ruminal ammonia N levels exceeded 5 mg per 100 ml of rumen ingesta. Satter and Roffler concluded, therefore, that NPN added to diets

containing more than 13% crude protein would not be utilized. They suggested that NPN is approximately equal in efficacy to protein as a source of N in "typical dairy and feedlot diets" containing not more than 12-13% crude protein.

The efficacy of a dietary NPN supplement may be dependent on the amount of NPN supplied by other dietary ingredients. Waldo (1968) stated that the percentage of total N in feedstuffs, which is present as NPN, varies from 4-5% in corn grain and soybean seed to 60-75% in "unwilted silages". Johnson *et al.* (1967) reported that NPN comprised 33.5 - 55.4% of the total N in corn silage. If diets already contain high concentrations of NPN, the data of Satter and Roffler (1975) would suggest that supplementary protein would be more effective than equivalent amounts of supplementary NPN.

Concentrations of nutrients other than nitrogen in the diet

The use of NPN supplements in place of protein supplements, on an equal N basis, results in addition to the diet of lower quantities of those nutrients other than protein that are available from protein supplements. If comparisons are to be made between NPN and protein supplements, adjustments must be made to the NPN diets to ensure that they are equivalent with respect to these other nutrients.

Diets containing NPN as the major source of supplemental N may require supplemental sulphur, since protein is the major dietary source of sulphur. Sulphur is required by rumen microorganisms for the syntheses of sulphur-containing amino acids and the vitamins thiamine and biotin. The nitrogen:sulphur ratio in a diet should be no greater than 12-15:1 for cattle and 10:1 for sheep (Oltjen, 1973; Garrigus, 1970).

Rys and Krewlowska (1963) studied the effects of adding phosphorus and cobalt to a diet for lactating cows in which urea provided 30% of the nitrogen. The addition of phosphoric acid resulted in an insignificant increase in N retention and increased the level of N in the milk. The addition of cobalt to the diet increased N retention.

Adaptation to non-protein nitrogen

The suggestion has been made in a number of reports that the magnitude of response to dietary NPN sources has changed with time after initial provision of the NPN. Studies involving the use of propionamide (Repp *et al.*, 1955), urea (Oltjen *et al.*, 1969; Caffrey *et al.*, 1967) and biuret (Schröder and Gilchrist, 1969; Clemens and Johnson, 1973) have indicated that adaptation to these NPN compounds occurred.

Adaptation to NPN may occur at the level of the

tissues of the host animal or in the population of rumen microorganisms. Tissue adaptation has been suggested by McLaren (1960) while Lewis (1960), Virtanen (1966) and Caffrey *et al.* (1967) implicated changes in the rumen microbial population.

The extent of dietary protein degradation in the rumen

The effectiveness of NPN as compared to protein in ruminant diets may be influenced by the extent to which the protein is degraded in the rumen and, hence, the quantities of protein, peptides and amino acids that escape from the rumen and are utilized by the animal post-ruminally. The rumen, being anaerobic, is predicted to impose definite limitations on microbial synthesis. Aerobic microorganisms incorporate 60-70% of carbohydrate substrate into cell synthesis (Hungate, 1966). Van Nevel *et al.* (1975) estimated that anaerobic microorganisms in the rumen incorporated 30% of digested carbohydrate. Thus, the potential yield of microbial protein as a percentage of ingested carbohydrate is comparatively low for the ruminant. Mathison and Milligan (1971) estimated that 1.7 - 2.6 g of N is assimilated into microbial protein for each 100 g of dry matter fermented.

Definite responses in wool growth were obtained with abomasal infusions of protein into sheep (Reis, 1969;

Robards, 1970; Dryden *et al.*, 1969) suggesting that amino acids resulting from the digestion of microbial protein may have been insufficient in quantities to meet requirements for maximal wool growth.

Preston (1972) fed steers basal diets composed of forage and molasses, supplemented with apparently adequate amounts of N in the form of urea. He then substituted fish meal for various levels of urea on an equal N basis. Fish meal was chosen because of its resistance to degradation in the rumen and its excellent amino acid profile. Increasing levels of fish meal provided increased rates of gain. While maximum response was obtained when fish meal contributed 50% of the total protein, Preston decided, because of the comparative costs of fish meal and urea, to use fish meal to supply 30% of the total protein.

Preston suggested that two separate N requirements be considered: the first being the amount of N required to maximize protein synthesis in the rumen and the second being the amounts of protein or amino acids which resist degradation in the rumen and which are necessary to supplement microbial protein in order to obtain high levels of production. He recommended that a comparatively inexpensive source of N such as urea be used to meet the first requirement.

Toxicity of non-protein nitrogen

Accumulation of ammonia in peripheral blood, resulting from the inability of the liver to convert all ammonia in the portal blood to urea, may cause toxicity (Chalupa, 1968; Visek, 1972). Although the amount of ammonia absorbed by the portal blood is proportional to the ruminal ammonia concentration, this may be modified by the pH of the rumen contents in that non-ionic ammonia (NH_3) is absorbed more readily than the ammonium ion (NH_4^+) and the pH will determine the ratio of NH_3 to NH_4^+ (Bloomfield *et al.*, 1963).

Toxic symptoms occur when the ammonia N levels in peripheral blood reach 1-4 mg/100 ml (Chalupa, 1968). Dinnings *et al.* (1948) found that ataxia occurred when the ammonia concentration in the systemic blood of steers reached 2.5 mg/100 ml. Alkalosis and death resulted from levels of 4 mg/100 ml. The symptoms of urea toxicity include dullness, laboured breathing, ataxia, excessive salivation, bloat, tetany and death (Word *et al.*, 1969; Chalupa, 1968).

Lewis (1960) stated that the toxicity encountered when excess amounts of urea are fed is due to a direct effect of the circulating ammonium ion level. The changes in acid-base balance were not sufficient, in his opinion, to cause the clinical signs of toxicity.

Gibson *et al.* (1974) injected mice with crystal-

line jackbean urease in dosages of 0.4 units of enzyme per mouse at 0 h and 12 h. The mice were killed 24 h after the first injections. Histological examination of brain tissue from mice showing symptoms of ammonia toxicity showed increases in number and size of astrocytic nuclei. In severe cases, spongy vacuolation of the neurophils was found.

Treatment for ammonia toxicity involves the administration of a weak acid (e.g. acetic acid) to neutralize rumen ammonia and to depress rumen pH, thereby decreasing the rate of absorption of rumen ammonia into the blood (Austin, 1967).

The level at which NPN is toxic to an animal is dependent on the method by which it is administered, the rate at which ammonia is released from the NPN supplement, the diet being fed and the general condition of the animal. Chalupa (1968) stated that the toxic level of urea for sheep, when given as a drench, is 20-30 g per 45 kg of body weight. Kromann *et al.* (1971) determined the "median lethal dose" (LD_{50}) of urea by administering various levels of urea via stomach tube to lambs fed a high energy, low protein diet. They estimated the LD_{50} of urea to be 28.5 g per 100 kg body weight. Dinnings *et al.* (1948) found that a drench containing 100 g or more of urea caused a very rapid rise in the blood ammonia level of a steer whereas the ad-

ministration of 400 g of urea via the feed caused no ill effects. The animal, however, took 5 h to consume the feed containing the urea.

Nix and Anthony (1965) reported that the injection of 200,000 IU of vitamin A intramuscularly apparently protected ewes against urea toxicity. However, the authors did not comment on the nature of the protective mechanism or the possible duration of the apparent protection. They obtained evidence to indicate that body condition has some effect on an animal's susceptibility to urea poisoning. Eight ewes were drenched with 27 g of urea per 45 kg body weight. The four ewes that were "fat and healthy" died; the four ewes that were "poor and physiologically depressed" survived.

The possible effects of energy level, protein level, age, period of fasting and level of urea on urea toxicity in sheep were examined by Kromann *et al.* (1971). Resultant blood ammonia concentrations were higher in the groups fed the high energy diet (85% concentrate) than in the group fed the low energy diet (85% roughage). Lambs were more susceptible than ewes to urea toxicity. A fasting period of 24 h had little effect on the incidence of toxicity.

Since ammonia resulting from hydrolysis of urea is causal in urea toxicity, supplementation with ammonium

salts would be expected to entail inherent danger of toxicity.

Fonnesbeck *et al.* (1975) reviewed numerous experiments in which biuret was used at high levels. They concluded that biuret *per se* could be considered as essentially non-toxic but that the toxicity of feed grade biuret is proportional to the amount of urea it contains. While biuret may be non-toxic to animals, it is toxic to plants (Tisdale and Nelson, 1971). Therefore, since biuret, when not hydrolyzed in the rumen, passes unchanged into the urine and, to a lesser extent, into the feces (Schröder, 1970), damage to pastures might result from the use of biuret as an N supplement for ruminants on pasture.

Assessment of non-protein nitrogen supplements: techniques and parameters

Traditional techniques for the evaluation of protein supplements include N balance, N retention, digestibility and growth trials. Other techniques have been designed or adapted specifically for the evaluation of NPN supplements.

In vitro methods

In vitro techniques have been used to compare and evaluate potential NPN supplements. Belasco (1956) incu-

bated rumen microorganisms *in vitro* with cellulose as the substrate and urea or other NPN compounds as a source of N and measured cellulose digestion and rate of ammonia release as criteria for comparing the compounds with urea. He considered the *in vitro* method to be very useful in screening large numbers of compounds.

Chalupa (1972) suggested that while *in vitro* techniques in which the rate of degradation of NPN is reflected in ammonia production are useful for preliminary evaluation of NPN compounds, the use of rumen fluid from animals not adapted to the particular compound may yield misleading results due to the lack of appropriate enzyme systems. He stated that better information on ammonia release rates might be obtained by using *in vivo* techniques.

Rate of ammonia production in the rumen

If ammonia produced from NPN is to be utilized by rumen microorganisms, carbon skeletons, resulting from carbohydrate fermentation, must be present (Chalupa, 1972). Fermentation products from many natural feedstuffs are produced at slow, sustained rates (Hungate, 1966). Thus, if dietary N is to be utilized efficiently, ammonia must be produced in the rumen at a rate similar to that of fermentation products since the absorption of ammonia from the rumen and, hence, the excretion of N in the urine are

related directly to the ruminal ammonia concentration (Lewis, 1957). Numerous researchers (Lewis, 1957; Huston *et al.*, 1974; Hembry *et al.*, 1975) have confirmed that there is a definite relationship between the efficiency with which supplemental N is used and the rate at which ammonia is produced from the supplement in the rumen.

Atwal, Young and Milligan (1971) described a technique for measuring rumen ammonia production *in vivo*. By determining patterns of ammonia accumulation in rumen fluid on the 1st, 8th and 15th days after mixed amides were first administered to sheep, the authors were able to show that the ability of the rumen microorganisms to degrade the amides was enhanced during the first week of the trial.

Milligan *et al.* (1972) used the same technique to assess the degradation of glucosyl-ureide in the rumen. They confirmed that glucosyl-ureide, on first administration, was degraded at a much slower rate than urea and that the rate at which ammonia was produced from this compound increased during the first seven days it was fed.

Blood urea concentration

Abou Akkada and El Sayed Osman (1967) obtained significant correlations ($P < 0.01$) among the blood urea, ruminal ammonia and N retention when leguminous forages were fed to desert sheep. As blood urea concentrations

increased N retention, as a percentage of N intake, decreased ($r = -0.87$).

Lewis (1957) investigated the potential of using blood urea concentration as a criterion for assessing the loss of N following the absorption of ammonia from the rumen in sheep. He first observed that an increase in rumen ammonia results in an increase in urinary N. He then determined that: blood urea levels were remarkably constant and without marked diurnal variations when constant diets were fed; blood urea values consistently reflected ruminal ammonia concentrations when samples were taken 4 h after feeding; changes in blood urea concentration reflect changes in rumen ammonia levels with a lag period of 4-6 h; and blood urea levels are influenced more by ruminal ammonia production than by total N intake. Lewis (1957) determined that when the level of ammonia in the rumen exceeds 25-30 mM, portal blood ammonia concentration increases rapidly and that, as a result, there would be increased urinary excretion of urea.

When Preston *et al.* (1965) fed diets containing 6.2 - 22.0% crude protein, they measured blood urea N (BUN) concentrations ranging from 2.7 - 32.9 mg/100 ml. The correlation between BUN and protein intake was high ($r = 0.986$). The following regression equation was derived: $y = 16.46 - 4.907x + 0.5032x^3$, where y = BUN(mg/100 ml)

and x = protein intake (g crude protein/$W^{\S}_{kg.}{}^{0.75}$). They suggested that with the type of diets fed in this experiment (50% cottonseed hulls plus varying proportions of soybean meal and corn), BUN concentrations in excess of 10 mg/100 ml would indicate that the dietary protein level was adequate. Tagari *et al.* (1964) found a significant negative correlation between blood urea concentration and N retention and a significant positive correlation between blood urea and ruminal ammonia levels. They considered that their data justified the use of blood urea as an index of protein utilization.

Egan and Kellaway (1971) evaluated several N metabolites as indices of N utilization in sheep fed roughages harvested at various stages of maturity. Blood and rumen fluid samples were taken at 0, 1 and 4 h after feeding. Plasma urea concentration was positively correlated with N intake and with the N apparently digested. They concluded that the relative efficiency of N utilization can be determined, at least for ranking purposes, by measuring plasma urea concentration before feeding and at various intervals after feeding. They felt, however, that the determination of plasma urea concentration on a

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§ Body weight.

single sampling where *ad libitum* feeding was used would allow prediction of urinary N values and N retention only within broad limits.

Ciszuk (1973) fed a variety of diets with a wide range of N contents to sheep and measured concentrations of ruminal ammonia and blood urea. He reported that blood urea was affected by ruminal ammonia, after a delay of several hours, but that there was no significant correlation between ruminal ammonia and blood urea concentrations.

The use of blood urea concentrations to predict, within a wide range, urinary N losses and N retention may be of value in large-scale feeding trials where conventional digestibility trials cannot be conducted. The determination of blood urea concentration also may be a rapid method by which the adequacy of dietary N concentrations can be assessed.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were conducted during the period 1973-1975 to evaluate two types of NPN compounds as potential feed supplements for ruminants. The compounds, N-glucosyl ureide (GU) and N-hydroxymethyl-N'-glucosyl ureide (MGU) were developed by staff of The Research Council of Alberta and The University of Alberta. GU is synthesized from glucose and urea while MGU is synthesized from glucose, urea and formaldehyde; when barley is used as the source of glucose, the products are designated as BBGU and BBMGU, respectively. Supplements containing these compounds were manufactured by The Research Council of Alberta and provided to The University of Alberta for investigation.

PART I

THE EVALUATION OF LIQUID SUPPLEMENTS CONTAINING GU AND MGU IN DIETS FOR GROWING CALVES

Introduction

Milligan *et al.* (1972) found that rumen microorganisms adapted to GU within 8 days of the time it was first fed. The rate of rumen ammonia accumulation from GU, after adaptation, was 14-22% of that from an isonitrogenous quantity of urea.

Preliminary testing of MGU indicated that it may be degraded in the rumen at a rate slower than that of urea. The use of one early preparation of this compound in a lamb feeding trial, however, caused symptoms of toxicity including stiffness of gait, muscle tremors, twitching of lateral abdominal musculature and apparent muscle weakness (Milligan, unpublished data). The symptoms first occurred 11 weeks after the beginning of the trial. Two animals died on day 81 of the trial. The remaining animals were removed from the experiment. The symptoms disappeared 7 to 10 days thereafter but reappeared when MGU was included again in the diet.

Post mortem examination of the two lambs showed

no evidence of lesions in the nervous system which could be associated with toxic or chemical products in the feed (Beck, 1973). Analyses for cholinesterase in the blood of sheep affected by the apparent MGU toxicity and normal sheep showed that cholinesterase levels in the affected sheep were somewhat higher than normal. Cholinesterase is an enzyme that destroys acetylcholine, a compound essential for the transmission of a nerve impulse at a neuromuscular junction immediately after the acetylcholine has caused the muscle fibre to generate an impulse (Guyton, 1969). Results of a feeding trial in which the preparation MGU was fed to rats suggested that MGU may cause elevated levels of cholinesterase which might explain the disorders encountered in the trial in which MGU was fed to sheep.

The purposes of this trial were to determine if MGU or GU would produce toxic symptoms in cattle when fed as part of a liquid supplement and to compare MGU, GU, urea and soybean meal as N supplements for calves fed diets low in digestible energy.

Materials and methods

The experiment was conducted at The University of Alberta's Ellerslie Test Station. Fifty steers (average initial body weight of 263 kg), of predominantly Hereford breeding, were purchased locally. On arrival at the

Station, each animal was vaccinated for blackleg and malignant edema and given an injection, intramuscularly, containing 500,000, 75,000 and 50 IU of vitamins A, D and E, respectively. The animals were fed a low quality timothy hay *ad libitum* for 14 days, then allotted randomly, on the basis of weight, to five treatment groups and ten pens, each pen (4 x 7.6 m) containing five animals. An open-front shed covered the feeding area in each pen. Wood shavings were used as bedding; water was supplied by automatic waterers. For the ten days prior to the commencement of the experiment, steam-rolled barley plus supplementary vitamins and minerals were fed, in addition to the hay, at a rate of 1.8 kg per head per day.

The experiment began on April 12, 1973. The animals were weighed on April 12, April 13 and weekly thereafter until the end of the trial when they were again weighed on August 1 and August 2. Initial and final weights were calculated as the means of the two successive weighings. The animals were weighed at approximately 0800 h and were denied access to feed and water for 14 h prior to that time.

Five treatments (control, urea, soybean meal, GU and MGU) were used (Table 1). Each group was fed a sufficient amount of a concentrate mixture to supply 1.8 kg per animal. Animals in the control, GU and MGU groups

Table 1. Treatments and rations used in the assessment of urea, soybean meal, GU and MGU as nitrogen supplements in diets fed to calves

	Control	Urea	Soybean meal	GU	MGU
Number of animals	10	10	10	10	10
Concentrate (kg/day)	1.8*	1.8	1.8	1.8*	1.8*
Blank liquid supplement (kg/day)	0.95	0.95	0.95	-	-
GU liquid supplement (kg/day)	-	-	-	0.93	-
MGU liquid supplement (kg/day)	-	-	-	-	1.13
Hay† *ad libitum*	+	+	+	+	+

* Control concentrate mixture.

† Timothy hay (10.1% crude protein, 42.8% acid detergent fibre, 0.71% calcium and 0.21% phosphorus on a dry matter basis).

received the control concentrate mixture while those in the urea and soybean meal groups were fed concentrate mixtures containing these supplements (Table 2). Liquid GU and MGU supplements (0.93 and 1.13 kg per head per day, respectively) were poured on the mixtures of hay and concentrate at the time of feeding. A blank liquid supplement was used for the control, urea and soybean meal groups in order to equalize the supplemental energy intake and physical form of the diets. The compositions of the liquid supplements are listed in Table 3. The concentrate-liquid supplement combinations used were estimated to contain similar concentrations of digestible energy using National Academy of Sciences - National Research Council (NAS-NRC) (1969) estimates of digestible energy concentrations of the components.

The chopped hay, which was fed *ad libitum* to each group, contained 10.1% crude protein, 42.8% acid detergent fibre, 0.71% calcium and 0.21% phosphorus on a dry matter basis.

The liquid supplements were manufactured by The Research Council of Alberta. Composite analyses of these products are listed in Table 3. The analyses of the liquid supplements were performed by The Research Council of Alberta using methods described by Wohllebe (1975).

Analyses of the hay and the concentrate mixtures

Table 2. Formulation and composition of concentrate mixtures fed in conjunction with liquid supplements and timothy hay

	Control	Urea	Soybean meal
Ingredients (%)			
Steam-rolled barley	95.2	89.7	59.1
Soybean meal (48.5% protein)	-	-	37.3
Urea (45% N)	-	5.5	-
Calcium phosphate*	2.0	2.0	0.8
Trace mineralized salt	1.0	1.0	1.0
Vitamin-mineral premix†	1.8	1.8	1.8
Chemical composition (%)			
Dry matter	83.4	78.8	84.5
Composition of dry matter (%)			
Crude protein	10.8	27.0	26.9
Calcium	0.50	0.51	0.27
Phosphorus	0.79	0.75	0.62
Digestible energy (Mcal/kg)§	3.48	3.32	3.52

* The calcium phosphate contained 18.5% calcium and 20.5% phosphorus.

† To supply 3400, 570 and 3 IU of vitamin A, D and E, respectively, per kg of concentrate and levels of 80, 21 and 40 ppm of zinc, copper and manganese, respectively, in the concentrate.

§ Estimated (NAS-NRC, 1969).

Table 3. Composite analyses of liquid supplements

	GU*	MGU†	Blank
Components (%)			
Dry matter	78.5	73.5	69.0
Unreacted glucose	6.7	4.3	-
Invert sugar	-	-	62.7
GU*	21.5	8.2	-
DGU§	10.0	11.2	-
Formaldehyde	-	1.0	-
MGU†	-	11.0	-
Partial elemental composition (%)			
Nitrogen	4.8	3.9	-
Calcium	1.6	1.5	1.7
Phosphorus	1.0	0.84	0.90
pH	4.6	7.9	3.9

* N-glucosyl ureide.

† N-hydroxymethyl-N'-glucosyl ureide.

§ N, N'-diglucosyl ureide.

were undertaken by the Agricultural Soil and Feed Testing Laboratory (ASFTL), Alberta Agriculture, Edmonton, using adaptations of standard Association of Official Agricultural Chemists (AOAC) methods as set out in the Agricultural Soil and Feed Testing Laboratory Manual (1975).

Blood samples were collected from each of the animals 6 h after feeding on days 43 and 111 of the experiment. Approximately 15 ml of blood was taken from the jugular vein using 20-gauge needles and vacuum tubes containing heparin. The samples were placed immediately in ice, transported to the laboratory and centrifuged at 671 x g for 25 min in accordance with standard procedures. The plasma was withdrawn and stored at approximately -25C for 14 days prior to analysis. Analysis for plasma urea nitrogen (PUN) was carried out using the method of Fawcett and Scott (1960) as adapted for use with the Technicon Auto-Analyzer (Agricultural Soil and Feed Testing Laboratory Manual, 1975). Samples of whole blood from five animals fed the control diet and five animals fed the MGU diet were forwarded to the Veterinary Laboratory, Alberta Agriculture, Edmonton, where cholinesterase analyses were undertaken, using the method described by Williams *et al.* (1957).

The data were analyzed statistically using a program (AOV5) available through The University of Alberta Computing Centre. Means were compared by the use of

Duncan's multiple range test (Steel and Torrie, 1960). A probability of 0.05 was used as the point of significance.

Results and discussion

Animals fed the control, urea, soybean meal, GU and MGU diets gained at average rates of 0.94, 0.85, 0.95, 0.89 and 0.75 kg per head per day, respectively, (Table 4). Statistical analysis showed that there were no significant differences in rates of gain among the control, soybean meal, urea and GU groups. The average daily gain of steers fed MGU, however, was significantly lower than the average daily gains for the control, soybean meal, urea and GU groups.

While treatment had no significant effect on the amount of hay consumed daily, there were significant differences in daily dry matter (DM) intakes (Table 4). The daily DM intakes of the control, soybean meal and urea groups (6.89, 6.94 and 7.07 kg, respectively) did not differ from each other. There were no significant differences between the daily DM intakes of the control group (6.89 kg), the soybean meal group (6.94 kg) and the MGU group (6.82 kg). Steers fed GU consumed less DM per day (6.71 kg) than steers fed the control, urea and soybean meal diets. The differences in daily DM consumption between the GU group (6.71 kg) and the MGU group (6.82 kg)

Table 4. Effects of liquid nitrogen supplements on the performance of steers fed timothy hay

	Control	Urea	Soybean meal	GU	MGU	SE[1]
Number of animals	10	10	10	10	10	
Feeding period (days)	111	111	111	111	111	
Average initial weight (kg)	263	262	264	265	261	2.29
Average final weight (kg)	367	357	370	364	344	3.59
Average daily gain (kg)	0.94*a*	0.85*a*	0.95*a*	0.89*a*	0.75*b*	0.02
Average hay intake (kg/day)*	4.70*a*	4.92*a*	4.76*a*	4.70*a*	4.60*a*	0.04
Average dry matter intake (kg/day)*	6.89*abc*	7.07*a*	6.94*ab*	6.71*c*	6.82*bc*	0.04
Average dry matter/kg of gain (kg)*	7.33*a*	8.32*ab*	7.33*a*	7.54*a*	9.09*b*	0.17
Average crude protein intake (g/day)†	631	883	862	907	894	

a, *b*, *c* Means followed by different letters in the same row are significantly different ($P < 0.05$).

* Dry matter basis.

† g N x 6.25.

[1] Standard error of the mean.

were not significant.

The feed:gain ratio for the MGU group (9.09) was significantly higher than those of the control, soybean meal and GU groups (7.33, 7.33 and 7.54, respectively) but not significantly different from that of urea (8.32) (Table 4). The urea group did not differ, in terms of feed:gain ratio, from the control, soybean meal and GU groups.

There was obviously no positive response, in terms of performance, to supplemental N in this experiment. The control group, however, gained at a rate that was higher than expected. The control group gained at a mean rate of 0.94 kg per day and consumed an average of 0.63 kg of protein (g N x 6.25) daily. The mean body weight of the animals in the control group at the mid-point of the trial was 315 kg. Predicted rates of gain for an animal weighing 300 kg and consuming 0.63 kg of protein per day and nutritionally adequate quantities of digestible energy, vitamins and minerals, range from 0.38 kg per day (NAS-NRC, 1971) to 0.80 kg per day (Agricultural Research Council, 1965). The actual rate of gain achieved by the control group was, therefore, 0.14 - 0.56 kg higher than would be predicted. The reason for this anomaly is not known.

The significantly lower rate of gain of the MGU group was not expected. The animals exhibited no signs of

toxicity, or irregular behavior. The mean cholinesterase levels in the MGU animals did not differ significantly from those in the control group (Table 5). Date of sampling had no significant effects on cholinesterase levels in the control and MGU groups. Previous work (Milligan, unpublished data) suggested that the toxic effects of one preparation of MGU might have been caused by or related to elevated cholinesterase levels. The depression in gain due to the feeding of MGU was not related to apparent toxic symptoms or cholinesterase levels in this experiment.

The PUN concentrations reflected, to some extent, the differences in N intake (Table 6). On each sampling date, the mean PUN concentrations of the MGU and control groups did not differ from each other but were significantly lower than the PUN concentrations of the urea, soybean meal and GU groups. The PUN concentrations of the urea, soybean meal and GU groups did not differ significantly at either sampling.

On the basis of the low concentrations of PUN for the control group (3.57 mg/100 ml on day 43 and 7.22 mg/100 ml on day 111), supplementation of the diet with N could be expected to increase average daily gain. Preston *et al.* (1965) suggested that concentrations of BUN in excess of 10 mg/100 ml would indicate that the dietary protein level was adequate. PUN concentrations

Table 5. Mean cholinesterase levels in whole blood from animals fed MGU or no supplemental nitrogen at two sampling dates*

Date of sampling	Number of animals	Cholinesterase (ΔpH/h) Control	MGU	SE[1]
Day 43	5	2.01	1.99	0.06
Day 111	5	1.95	1.90	0.05

* There were no significant differences between treatments or dates of sampling.

[1] Standard error of the mean.

Table 6. Mean plasma urea nitrogen concentrations in steers fed diets containing urea, soybean meal, GU, MGU or no supplemental nitrogen at two sampling dates in trial 1

Date of sampling	Plasma urea nitrogen (mg/100 ml)					
	Control	Urea	Soybean meal	GU	MGU	SE[1]
Day 43	3.57*a*	11.46*b*	10.88*b*	11.54*b*	8.50*c*	0.53
Day 111	7.22*a*	14.72*b*	13.68*b*	14.09*b*	10.66*c*	0.47

a, *b*, *c* Means followed by different letters in the same row are significantly different ($P < 0.05$).

[1] Standard error of the mean.

are approximately 10% higher than BUN concentrations (Ciszuk, 1973). Thus, concentrations of PUN lower than 11 mg/100 ml should, according to Preston *et al.* (1965), be indicative of levels of dietary protein that are insufficient to support maximal growth.

The PUN concentrations for the control group in this experiment suggested that the average daily gains in this group would be much lower than those actually observed. Other researchers have indicated relationships between PUN and protein content of the diet or response to supplemental protein. Young *et al.* (1973) fed a basal diet consisting almost entirely of ground ear corn and containing 6.98% protein, on an air-dry basis. Supplemental soybean meal and urea were added to the basal diet resulting in diets containing 11.0% and 10.0% protein. PUN concentrations on day 56 were 6.00, 9.20 and 10.26 mg/100 ml for the control, soybean meal and urea groups, respectively; concentrations on day 112 were 4.94, 10.77 and 8.87 mg/100 ml. Average daily gains during the trial were 0.96, 1.10 and 1.00 kg for the control, soybean meal and urea groups, resppctively. Macleod *et al.* (1975) fed diets high in digestible energy and obtained a response to supplemental protein during the first 84 days of the trial. Mean PUN concentrations were 10.5 mg/100 ml for the group receiving supplement and 6.9 mg/100 ml for the group fed the control

diet.

The concentrations of PUN for the urea, soybean meal and GU groups (11.46, 10.88 and 11.54 mg/100 ml, respectively, on day 43 and 14.72, 13.68 and 14.09 mg/100 ml, respectively, on day 111) would suggest, according to Preston *et al.* (1965), that protein requirements were met throughout the feeding period. The intermediate PUN concentrations from the MGU group (8.50 and 10.66 mg/100 ml on days 43 and 111, respectively) might suggest that MGU was degraded more slowly than the other supplements.

The lack of response to supplemental N obviates comparisons of the supplements. MGU depressed gain and did not raise blood urea to the same extent as the other N supplements. The experiment demonstrated, however, that GU and MGU may be fed for 111 days without causing any obvious toxic effects.

PART II

THE ASSESSMENT OF SOLID BBGU AS A NITROGEN SUPPLEMENT IN CATTLE DIETS CONTAINING LOW OR HIGH CONCENTRATIONS OF DIGESTIBLE ENERGY

Introduction

Research conducted by staff of The University of Alberta and The Research Council of Alberta on slow release NPN supplements led to the development of two solid products, barley-based N-glucosyl-N'-hydroxymethyl ureide (BBMGU) and barley-based N-glucosyl ureide (BBGU). These are preparations of MGU and GU for which barley was used as the source of glucose. Both preparations contain the nutrients present in barley in addition to the N from urea.

A liquid supplement, which contained MGU and which was made from invert sugar, urea and formaldehyde, had been found to depress average daily gain when it was fed to growing calves (Part I). A decision, therefore, was made to concentrate on the development and investigation of GU in its barley-based solid form. The BBGU product appeared to have physical properties desirable for a commercial supplement: it had a high content of N, may be comparatively inexpensive to manufacture and, on

very preliminary examination, was consumed readily by sheep.

This study was designed to compare BBGU with soybean meal as an N supplement for diets of low or high digestible energy content using rate of gain of cattle, feed:gain ratio, PUN and carcass characteristics as criteria of assessment.

Materials and methods

The experiment was conducted at The University of Alberta's Ellerslie Test Station. Forty-eight steers (average initial body weight of 239 kg), of predominantly Hereford breeding, were purchased locally. On arrival at the Station, each animal was vaccinated for blackleg and malignant edema and given an injection, intramuscularly, containing 500,000, 75,000 and 50 IU of vitamins A, D and E, respectively. The animals were introduced to a diet consisting of chopped straw *ad libitum* plus approximately 1 kg of steam-rolled barley per head per day for a period of 14 days and then allotted randomly, on the basis of weight, to three treatment groups and ten pens, each pen containing a maximum of five animals. The facilities used to house the animals were similar to those described in Part I. However, the pens were adapted to facilitate individual feeding by the installation of five headgates

at the feed-bunks of each pen. Water was supplied by automatic waterers. Wood shavings were used as bedding.

Each animal was weighed on October 30 and 31, 1973, after being without feed and water for 14 h immediately prior to weighing, and the mean of the two weights was considered to be that animal's weight at the beginning of the experiment. The animals were weighed weekly throughout the trial at approximately 0800 h and were denied access to feed and water for 14 h immediately prior to that time.

The experiment was divided into two periods. Diets containing low concentrations of digestible energy were fed during the first, or growing, period. The energy levels of the diets were increased during the second, or fattening, period. The diets fed to the control and two experimental groups contained BBGU, soybean meal or no supplemental N, respectively, throughout the first and second periods.

Period 1: Grower diets

Steers, weighing an average of 239 kg at the beginning of the trial, were individually fed diets consisting of 66.7% oat straw and 33.3% concentrate. The steers were locked in headgates at feeding time; the ration for each animal was fed in separate wooden boxes fitted

into the feed bunks. Formulations and compositions of the diets are listed in Table 7.

The animals became accustomed to entering their own headgates soon after the experiment began. The steers were locked in the headgates for 2.5 h in the morning and 2.5 h in the afternoon, during which times the diets were provided to voluntary consumption. Any feed remaining in the bunks was weighed weekly, mixed with new feed and fed to the animals.

The diets were fed for 154 days. On days 65 and 151, 15 ml blood samples were collected from each of the animals by jugular vein puncture using 20-gauge needles and vacuum tubes containing heparin. The samples were placed immediately in ice, transported to the laboratory and centrifuged at 671 x g for 25 min in accordance with standard procedures. The plasma was withdrawn and stored at approximately -25C for subsequent analyses. Analysis for PUN was done within 14 days of collection of the samples using the method of Fawcett and Scott (1960) as adapted for use with the Technicon Auto-Analyzer (Agricultural Soil and Feed Testing Laboratory Manual, 1975).

The low energy diets were fed for 154 days. Means of individual weights collected on days 153 and 154 were considered to be the final weights of period 1 and the initial weights of period 2.

Table 7. Formulation and composition of diets fed to steers during period 1 of a trial comparing BBGU to soybean meal as a nitrogen supplement

	Control	BBGU	Soybean meal
Ingredients (%)			
Oat straw	66.7	66.7	66.7
Concentrate	33.3	33.3	33.3
Concentrate (%)			
Steam-rolled barley	93.7	61.5	52.2
Soybean meal (48.5% protein)	-	-	42.1
BBGU*	-	32.8	-
Ground limestone	1.6	2.4	1.5
Calcium phosphate[T]	1.4	0.1	1.0
Trace mineralized salt	1.0	1.0	1.0
Vitamin premix[†]	2.2	2.2	2.2
Chemical composition (%)			
Dry matter	84.2	84.5	84.5
Composition of dry matter (%)			
Crude protein	7.0	10.5	11.0
Calcium	.36	.40	.38
Phosphorus	.25	.31	.26
Digestible energy (Mcal/kg)[§]	2.83	2.73	2.82

* Barley-based N-glucosyl ureide (53% crude protein, 0.1% calcium, 1.3% phosphorus).

[T] The calcium phosphate contained 18.5% calcium and 20.5% phosphorus.

[†] To supply 5070, 838 and 5.1 IU of vitamin A, D and E, respectively, per kg of total diet.

[§] Estimated (NAS-NRC, 1969).

Period 2: Fattening diets

The diets fed during this period contained 85% concentrate and 15% oat straw. The concentrate mixtures included either BBGU, soybean meal or no supplemental N (Table 8).

The animals were fed individually. The feeding procedure was similar to that used in period 1. The steers were provided with increasing amounts of the diets until maximum consumption was reached, approximately 21 days after the beginning of the period. Amounts fed throughout the remainder of the period were adjusted as often as was necessary to provide sufficient feed for maximum consumption. The concentrate:straw ratio remained the same throughout the period.

Blood samples were taken and processed on day 284 and analyzed for PUN within 14 days using the procedures employed in period 1.

The animals were marketed when they reached 450 - 475 kg live-weight. Carcass measurements were made by Agriculture Canada personnel. The rumen walls and livers from the steers were examined and the numbers condemned were tabulated.

The data from this experiment were analyzed using an analysis of variance program (AOV5) available from The University of Alberta Computing Centre. Means

Table 8. Formulation and composition of diets fed to steers during period 2 of a trial comparing BBGU to soybean meal as a nitrogen supplement

	Control	BBGU	Soybean meal
Ingredients (%)			
Oat straw	15	15	15
Concentrate	85	85	85
Concentrate (%)			
Steam-rolled barley	97.8	91.9	91.9
Soybean meal (48.5% protein)	-	-	5.9
BBGU*	-	5.9	-
Ground limestone	1.2	1.2	1.2
Trace mineralized salt	0.59	0.59	0.59
Vitamin premix†	0.35	0.35	0.35
Sulphur (elemental)	0.06	0.06	0.06
Chemical composition (%)			
Dry matter	87.8	87.4	88.3
Composition of dry matter (%)			
Crude protein	10.1	11.6	11.8
Calcium	0.44	0.45	0.63
Phosphorus	0.38	0.38	0.38
Digestible energy (Mcal/kg)§	3.32	3.33	3.31

* Barley-based N-glucosyl ureide (53% crude protein, 0.1% calcium, 1.3% phosphorus).

† To supply 5944, 1165 and 5.94 IU of vitamin A, D and E, respectively, per kg of total diet.

§ Estimated (NAS-NRC, 1969).

were compared using Duncan's multiple range test (Steel and Torrie, 1960). A probability of 0.05 was used as the point of significance. Analysis of covariance was used to adjust means of average daily gains and average daily DM intakes using initial body weight in period 2 as a co-variate.

Analyses of composite mixtures of the diets were carried out by the ASFTL using adaptations of standard AOAC methods (Agricultural Soil and Feed Testing Laboratory Manual, 1975).

Results and discussion

The animals were marketed during the period of August 20 to October 16, 1974. The total feeding period for the individual animals, therefore, varied from 294 - 391 days. One animal was removed from the trial because it obviously had not been properly castrated.

The average growth rates, DM intakes and feed:gain ratios for the entire experiment are listed in Table 9. Protein supplementation did not influence rate of gain significantly. The average daily gains for the control, BBGU and soybean meal groups were 0.64, 0.70 and 0.68 kg, respectively.

There was a significant interaction between feeding period and protein supplementation ($P < 0.05$)

Table 9. Performance of steers fed diets containing BBGU, soybean meal or no supplemental nitrogen

	Control	BBGU	Soybean meal	SE[1]
Number of animals	15	16	16	
Average initial weight (period 1) (kg)	239.0	240.4	238.6	8.50
Average initial weight (period 2) (kg)	275.8*a*	286.6*ab*	296.8*b*	3.14
Average final weight (kg)	456.3*a*	472.4*b*	465.4*ab*	2.64
Average daily gain (kg)*	0.64*a*	0.70*a*	0.68*a*	
Average dry matter intake (kg/day)*	6.97*a*	7.71*b*	7.54*b*	
Average dry matter/kg gain (kg)*	16.46*a*	14.88*ab*	12.85*b*	
Average days on test (period 1)	154	154	154	
Average days on test (period 2)	177.8	168.8	171	4.64

a, *b* Means followed by different letters in the same row are significantly different ($P < 0.05$).

* Mean of period 1 and period 2.

[1] Standard error of the mean.

(Table 10A). During period 1, the steers fed soybean meal gained significantly more rapidly than did those fed the control diet. The rate of gain of the BBGU group was not significantly different from those of the other groups. During period 2, the BBGU group gained slightly, but not significantly, more rapidly than the control group (1.11 vs. 1.04 kg per head per day). There was no significant difference between the average daily gains of the soybean meal and control groups (0.99 and 1.04 kg, respectively) while the difference between the average daily gains of the BBGU and soybean meal groups was significant ($P < 0.05$).

Van Slyke *et al.* (1971), Frietag *et al.* (1968) and Lassiter *et al.* (1958) found that when diets low in digestible energy were fed, the addition of protein supplements provided higher rates of gain than did the addition of NPN. NPN supplements were as effective as protein supplements in diets high in digestible energy according to Lowrey and McCormick (1969) and Oltjen *et al.* (1965). MacLeod *et al.* (1975), however, found that steers weighing 408 kg or more did not respond to supplemental protein added to a shelled corn-corn silage diet which contained 9.2% crude protein (DM basis). Therefore, one might not expect a response, in average daily gain, to supplemental N in period 2 as the control diet contained 10.1% crude protein.

Table 10A. Interaction of feeding period and nitrogen supplements on average daily gain (kg)

Period	Nitrogen supplement			
	Control	BBGU	Soybean meal	SE[1]
1	0.25*a*	0.30*ab*	0.37*b*	0.01
2*	1.04*cd*	1.11*c*	0.99*d*	0.02

a, b, c, d Means followed by different letters are significantly different ($P < 0.05$).

[1] Standard error of the mean.

* Means were adjusted by analysis of covariance using initial body weight in period 2 as a covariate.

The average daily DM intakes for the BBGU and soybean meal groups (7.71 and 7.54 kg, respectively) were significantly higher than that of the control group. The interaction between period and protein supplementation was significant ($P < 0.05$) (Table 10B). During period 1, the steers fed soybean meal ate slightly, but not significantly, more than those fed BBGU while both groups consumed significantly more DM than the control group. In period 2, the control, BBGU and soybean meal groups consumed averages of 8.49, 9.41 and 8.71 kg of DM per head per day, respectively. Animals fed BBGU consumed significantly more DM than either the control or soybean meal groups. The difference in DM intake between the control and soybean meal groups was not significant. The differences in DM intake may explain why animals fed BBGU in period 2 gained significantly more rapidly than the soybean meal group and tended to gain faster than the control group.

The average feed:gain ratios (kg DM/kg gain) during the experiment were 16.46 for the control group, 14.88 for the BBGU group and 12.85 for the soybean meal group. The control group did not differ significantly from the BBGU group in this respect while the BBGU group did not differ significantly from the soybean meal group. A significant interaction ($P < 0.05$) between period and protein supplementation occurred (Table 10C). The feed:gain

Table 10B. Interaction of feeding period and nitrogen supplements on average daily dry matter intake (kg)

	Nitrogen supplement			
Period	Control	BBGU	Soybean meal	SE[1]
1	5.63*a*	6.01*b*	6.20*b*	0.14
2*	8.49*c*	9.41*d*	8.71*c*	0.21

a, *b*, *c*, *d* Means followed by different letters are significantly different ($P < 0.05$).

[1] Standard error of the mean.

* Means were adjusted by analysis of covariance using initial body weight in period 2 as a covariate.

Table 10C. Interaction of feeding period and nitrogen supplements on feed:gain ratio (kg dry matter/kg gain)

Period	Nitrogen supplement			
	Control	BBGU	Soybean meal	SE[1]
1	24.74*a*	21.23*b*	16.77*c*	1.02
2*	8.18*d*	8.53*d*	8.93*d*	0.13

a, b, c, d Means followed by different letters are significantly different ($P < 0.05$).

[1] Standard error of the mean.

* Means were adjusted by analysis of covariance using initial body weight in period 2 as a covariate.

ratios for the control, BBGU and soybean meal groups all differed significantly from each other in period 1 but did not differ from each other in period 2.

The increased response to BBGU in period 2 may be due partly to an effect of BBGU on the palatability of the high-concentrate diet. The mean glucose content of the BBGU used in the experiment was 0.74% (Research Council of Alberta, unpublished data). The product *per se* was apparently quite palatable in very preliminary trials with sheep, possibly due to the unreacted glucose.

The data reported by Fox *et al.* (1972) may be relevant to the results reported herein. Fox *et al.* (1972) fed groups of steers maintenance diets over periods of 154 or 190 days and then fed fattening diets, high in digestible energy, until the animals reached 364 or 454 kg live-weight. A comparative slaughter technique was used to determine the amount of fat and protein deposited during the experiment. The steers fed the compensatory diets (a maintenance and, later, a fattening diet) had, at 364 kg live-weight, carcasses higher in protein and lower in fat than those of the control group, which was fed only the fattening diet. Body composition at 454 kg was not affected by treatment. The authors concluded that the steers fed the compensatory diets deposited more protein and less fat than the control group early in the fattening period but deposited more fat

than the control steers during the latter part of the fattening period. The steers in the compensatory group consumed more DM, gained more rapidly and had lower ratios of feed:gain than the control group during the fattening period. The soybean meal group, which consumed the most DM and gained the most rapidly in period 1, gained the least rapidly in period 2. The animals fed the control and BBGU diets may have exhibited compensatory gain, to some degree, in period 2. Unfortunately, carcass composition data were not collected in this experiment. Such data may have shown whether there were, in fact, differences in body composition at slaughter.

PUN concentrations for the three treatment groups are found in Table 11. Assuming that PUN concentrations in excess of 11 mg/100 ml indicate that dietary protein concentrations are sufficient for maximum rates of growth (Preston, 1965; Ciszuk, 1973), the amounts of N consumed by the BBGU and soybean meal groups were apparently adequate at all stages of the experiment and the quantity supplied by the control diet was inadequate. The PUN concentrations in the BBGU and soybean meal groups were significantly higher ($P < 0.05$) than those of the control group on all sampling dates. The

Table 11. Plasma urea nitrogen concentrations in steers fed diets containing BBGU, soybean meal or no supplemental nitrogen at three sampling dates

Date of sampling	Plasma urea nitrogen (mg/100 ml)			
	Control	BBGU	Soybean meal	SE[1]
Day 65 (period 1)	3.61*a*	14.29*b*	15.34*b*	0.84
Day 151 (period 1)	3.30*a*	16.95*b*	16.15*b*	0.98
Day 284 (period 2)	7.58*a*	13.81*b*	12.10*c*	2.16

a, *b*, *c* Means followed by different letters in the same row are significantly different ($P < 0.05$).

[1] Standard error of the mean.

PUN concentrations in the BBGU and soybean meal groups did not differ significantly from each other on days 65 and 151. On day 284, the PUN concentration of the BBGU group (13.81 mg/100 ml) was significantly higher than that of the soybean meal group (12.10 mg/100 ml) possibly reflecting a difference in average DM consumption (Table 10B).

The PUN concentration of the control group on day 284 (7.58 mg/100 ml) was considerably higher than the concentrations on days 65 and 151 (3.61 and 3.30, respectively). This difference in PUN is likely a reflection of the difference in crude protein content of the control diet fed in period 1 (7.0% on a DM basis) and the control diet fed in period 2 (10.1% on a DM basis). PUN concentrations indicate that neither diet met protein requirements.

Carcass measurements, obtained on 41 of the 48 animals marketed, are listed in Table 12. Treatment had no significant effects on carcass traits. The fat cover measurements (Table 12) and the carcass grades (Table 13) suggest that steers fed BBGU or soybean meal had a slightly higher degree of finish than those fed the control diet.

There were no discernible differences among the treatment groups in numbers of livers condemned. One liver from the soybean meal group was condemned while none of the livers from the control and BBGU groups was rejected.

BBGU, therefore, provided responses in growth

Table 12. Mean carcass measurements from animals fed diets containing BBGU, soybean meal or no supplemental nitrogen

	Control*	BBGU*	Soybean meal*	SE[1]
Number of carcasses examined	14	13	14	
Dressing percentage	54.9	55.7	56.1	0.30
Area of rib eye (cm^2)	60.9	61.1	62.0	1.12
Fat cover (cm)	1.58	1.80	1.79	0.06
Carcass weight (kg)	250.8	260.3	259.7	1.92

* Analysis of variance showed that treatments had no significant effects on carcass characteristics.

[1] Standard error of the mean.

Table 13. Carcass grades of animals fed diets containing BBGU, soybean meal or no supplemental nitrogen

Treatment	Number of animals within each grade			
	A1*	A2*	A3*	A4*
Control	4	8	1	1
BBGU	2	3	8	-
Soybean meal	1	10	2	1

* Agriculture Canada Beef Grades.

rate, DM intake and efficiency of feed utilization that were at least as good as those resulting from soybean meal. The increase in feed consumption obtained with BBGU may point to an important palatability factor that should be evaluated in future trials. BBGU had no apparent deleterious effects on animal behavior, health or carcass characteristics.

PART III

DIGESTIBILITY AND NITROGEN BALANCE STUDY

Introduction

Nitrogen balance studies are considered to be valuable in the assessment of NPN supplements (McLaren, 1964). The effectiveness of an NPN supplement is dependent on the percentage of N in the supplement that is retained by the animal. Since energy is often a limiting factor in ruminant diets, determination of the level of digestible energy in a dietary supplement is essential to any comprehensive evaluation of that supplement.

The purpose of this study was to determine the digestibility of energy and N of BBGU and BBMGU and to estimate the percentages of N of these products which were retained when the products were eaten by sheep.

Materials and methods

Six mature crossbred wether sheep, weighing an average of 53.6 kg, were used in the experiment. Each animal received an intramuscular injection containing 250,000, 37,500 and 25 IU of vitamins A, D and E, respectively, at the beginning of the trial.

Three diets consisting of alfalfa pellets (100%), alfalfa pellets (80%) plus BBGU (20%) and alfalfa pellets (80%) plus BBMGU (20%) were used (Tables 14 and 15) during two feeding periods, each consisting of a 14-day adaptation interval and a 7-day collection period. Each diet was fed to two sheep during the first period. After the first period, the diets were switched so that, in total, each diet was fed to four sheep.

The diets supplied maintenance levels of digestible energy as estimated by the formula (NAS-NRC, 1957):

$$DE(kcal) = 2 \times 70\ W_{kg}^{0.75}.$$

The animals were housed indoors (average temperature of 21C) in metal metabolism crates. Urine was collected daily in plastic pails containing 40 ml of 25% (v/v) sulphuric acid. The volume of urine was recorded and 10% by volume was combined with previous samples from the same animal and frozen for subsequent analysis. Feces were collected daily on mesh screens, weighed and 5% retained, dried at 100C for 16 h and added to samples taken previously from the same animal fed the same diet. The urine samples were thawed and analyzed for N using the Kjeldahl technique (AOAC, 1970). Composite feed samples were analyzed for moisture, N, calcium, phosphorus, acid detergent fibre and ether extract using standard AOAC techniques as adapted by the ASFTL (Agricultural Soil and Feed Testing Laboratory

Table 14. Analyses of feed ingredients used in nitrogen balance trial

	Alfalfa pellets	BBGU	BBMGU
Chemical composition (%)*			
Nitrogen	2.08	9.59	8.87
Calcium	1.28	0.11	0.11
Phosphorus	0.24	1.51	1.38
Ether extract	2.6	1.1	0.8
Acid detergent fibre	39.5	7.6	10.2
Gross energy (cal/g)	4460	4090	3980

* Dry matter basis.

Table 15. Composition and analyses of diets fed in digestibility trial

	Control	BBGU	BBMGU
Ingredients (%)			
Alfalfa	100	80	80
BBGU	-	20	-
BBMGU	-	-	20
Chemical composition (%)			
Dry matter	90.9	91.3	91.1
Composition of dry matter (%)			
Nitrogen	2.08	3.57	3.44
Calcium	1.28	1.02	1.02
Phosphorus	0.24	0.49	0.47

Manual, 1975). Moisture and N analyses also were carried out on the dried fecal samples. A Parr adiabatic oxygen bomb calorimeter was used to determine the gross energy contents of all feed and fecal samples.

The data were analyzed by means of analysis of variance, using a program (AOV5) available through The University of Alberta Computing Centre. Means were compared through the use of Duncan's multiple range test (Steel and Torrie, 1960). A probability of 0.05 was used, in all cases, as the point of significance.

The estimated digestibility coefficients, N retention and digestible energy values for BBGU and BBMGU were calculated using the method described by Crampton and Harris (1969).

Results and discussion

The addition of BBGU or BBMGU to the basal diet significantly increased apparent digestibilities of DM, energy and N (Table 16). The means of the digestibilities of N in the BBGU and BBMGU diets were significantly higher than that of the N in the basal diet. The N retention and N balance for the control group were negative, indicating a net loss of N when this diet was fed (Table 16). The apparent energy digestibility of the BBGU diet (59.7%) was significantly higher than that of the BBMGU diet (57.5%).

Table 16. Mean apparent digestibility coefficients for dry matter, energy and nitrogen, mean nitrogen retention and nitrogen balance values for diets fed in digestibility trial

	Control	BBGU	BBMGU	SE[1]
Apparent digestibility of dry matter (%)	52.0*a*	58.7*b*	56.5*b*	0.98
Apparent digestibility of energy (%)	53.2*a*	59.7*b*	57.5*c*	0.93
Apparent digestibility of nitrogen (%)	38.3*a*	74.0*b*	68.6*b*	5.56
Nitrogen balance (g/day)	-4.89*a*	4.87*b*	6.40*b*	1.78
Nitrogen retention (%)	-20.6*a*	13.2*b*	18.4*b*	6.24

a, *b* Means followed by different letters in the same row are significantly different ($P < 0.05$).

[1] Standard error of the mean.

The estimated mean coefficient of N digestibility for BBGU (105.5% ± 5.64) was significantly higher than that for BBMGU (75.6% ± 5.56) (Table 17). The inclusion of BBGU in the diet may have increased the utilization of N from the alfalfa, thus resulting in an estimated coefficient of N digestibility for BBGU which exceeded 100%. BBGU and BBMGU did not differ significantly in apparent DM digestibility, apparent energy digestibility, digestible energy (cal/g) nor in the proportion of N from these supplements which the animals retained. There were, however, apparent differences which would suggest that BBGU was superior in all respects to BBMGU (Table 17).

The digestible energy (DE) value for BBGU (3488 cal/g) is similar to that quoted by NAS-NRC (1969) for soybean meal (3527 cal/g) but lower than that quoted for barley grain (3792 cal/g). The DE value calculated for BBMGU (3002 cal/g) is similar to that suggested by NAS-NRC for rapeseed meal (3086 cal/g).

Table 17. Estimated digestibility coefficient, nitrogen retention and digestible energy values (±SE[1]) for BBGU and BBMGU*

	BBGU	BBMGU
Apparent digestibility of dry matter (%)	85.6 ± 3.89	74.5 ± 6.88
Apparent digestibility of energy (%)	85.3 ± 3.16	74.4 ± 6.81
Digestible energy (cal/g)	3488.0 ± 129.4	3002.4 ± 271.2
Apparent digestibility of nitrogen (%)	105.5*a* ± 5.64	75.6*b* ± 5.56
Nitrogen retention (%)	43.0 ± 9.41	27.4 ± 7.23

a, *b* Means followed by different letters in the same row are significantly different ($P < 0.05$).

[1] Standard error of the mean.

* Values estimated by difference, using method of Crampton and Harris (1969).

PART IV

AMMONIA ACCUMULATION IN THE RUMEN AFTER ADMINISTRATION OF BBGU, BBMGU, SOYBEAN MEAL AND UREA

Introduction

The efficiency of utilization of the N of an NPN supplement is related to the rate of ammonia accumulation in the rumen when the supplement is fed (Hembry *et al.*, 1975; Huston *et al.*, 1974). The rate at which ammonia passes into the portal blood is dependent on the ammonia concentration in the rumen (Lewis, 1957). Ammonia in the portal blood is converted to urea in the liver and then may be excreted via the kidney. If the capacity of the liver to convert ammonia to urea is exceeded, the resulting ammonia accumulation in peripheral blood could prove toxic to the animal (Chalupa, 1968). The efficiency of utilization of NPN by an animal may increase as the length of time during which it is fed increases (McLaren *et al.*, 1965; Oltjen, 1969) due, perhaps, to adaptive changes in the microbial population (Lewis, 1960) or in the tissues of the animal (McLaren, 1964).

This experiment was designed to evaluate and compare the rates of degradation of BBGU, BBMGU, urea and

soybean meal in the rumen and to determine the effects of adaptation on the rates at which these compounds result in ammonia accumulation.

Materials and methods

A 4 x 4 latin square experimental design was employed using four crossbred steers, with rumen *fistulae*, of 347 kg average initial body weight and four N sources including BBGU, BBMGU, urea and soybean meal. Each steer was fed 4.4 kg of oat straw and 2.2 kg of a concentrate mixture daily during the experiment. The concentrate mixtures contained either BBGU, BBMGU, urea, soybean meal or no supplemental N (Table 18). The control diet, which consisted of straw and the concentrate mixture without supplemental N, contained 6.5% crude protein on a DM basis. The equivalent crude protein concentration in each of the other diets was 12.4% on a DM basis. The concentrations of unreacted urea contained in the BBGU and BBMGU preparations used in this experiment were 2.83% and 0.17%, respectively.

The procedure used to assess the rate at which the N supplements were degraded was similar to that described by Atwal *et al.* (1971). Each steer was fed the control concentrate mixture for 13 days and a concentrate mixture containing one of the N supplements for the following 15 days. On days 1, 8 and 15 of the supplemented

Table 18. Formulation of diets fed in rumen ammonia release experiment

	Control	Soybean meal	Urea	BBGU	BBMGU
Ingredients (%)					
Oat straw	66.7	66.7	66.7	66.7	66.7
Concentrate	33.3	33.3	33.3	33.3	33.3
Concentrate (%)					
Steam-rolled barley	93.7	52.2	87.5	61.5	54.4
Soybean meal (48.5% protein)	-	42.1	-	-	-
BBGU†	-	-	-	32.8	-
BBMGUT	-	-	-	-	40.0
Urea	-	-	6.0	-	-
Ground limestone	1.6	1.5	1.6	2.4	2.3
Calcium phosphate§	1.4	1.0	1.7	0.1	0.1
Trace mineralized salt	1.0	1.0	1.0	1.0	1.0
Vitamin premix*	2.2	2.2	2.2	2.2	2.2

† Barley-based N-glucosyl ureide (60% crude protein, 0.1% calcium, 1.51% phosphorus on a dry-matter basis).

T Barley-based N-hydroxymethyl-N'-glucosyl ureide (55.4% crude protein, 0.1% calcium, 1.38% phosphorus on a dry-matter basis).

§ The calcium phosphate contained 18.5% calcium and 20.5% phosphorus.

* To supply 6160, 836 and 5.1 IU of vitamin A, D and E, respectively, per kg of total ration.

period, an amount of the supplement sufficient to supply 60 g of N (30 g of N in the case of urea) was mixed with 3.0 l of water (40C) and administered intraruminally (Table 19) through a rumen fistula at 0800 h. The animals were denied access to feed and water for 12 h immediately prior to dosing with the N supplements. Samples of rumen fluid were taken 15 min prior to the administration of the supplement and at pre-determined intervals up to 12.5 h after administration. Each sample (approximately 15 ml) was removed from the rumen by suction, filtered through a Surge syphon filter pad (No. 20137, Babson Bros. Ltd., Chicago, Ill.) into a 20 ml scintillation vial containing three drops of concentrated sulphuric acid and stored at -17C for subsequent analysis, in accordance with the experimental procedure used by Atwal *et al.* (1971). The ammonia concentrations in the samples were determined using an Orion ammonia electrode (Model 95-10, Orion Research Inc., Cambridge, Mass.) in conjunction with a Fisher Model 520 digital pH/ion meter. Five ml of rumen fluid were added to 45 ml of 0.2 M NaOH and the electrode potential of the mixture was measured. A standard curve prepared by determining the electrode potential of ammonium chloride solutions to which 0.2 M NaOH had been added and containing equivalents of 0.1 - 100 ppm NH_3 was used to determine the ammonia concentration in each sample of rumen fluid. The

Table 19. Amounts of NPN supplements and soybean meal administered intraruminally on each test day during rumen ammonia accumulation experiment

Supplement	Nitrogen content (%)	Supplement administered on test day (g)	Nitrogen added (g)
BBGU	7.99	751	60.0
BBMGU	7.84	765	60.0
Urea	45.00	67	30.0
Soybean meal	7.76	773	60.0

ammonia concentration in ppm was converted to an equivalent concentration (mM) in rumen fluid.

As a result of the latin square design, each animal was subjected to each of the four dietary treatments. Mean changes in ruminal ammonia concentration for each treatment and collection day were calculated and illustrated graphically. Due to an error in administration of the test dose, the data from one animal to which the urea was administered on day 8 were not used. Samples also were collected from each animal over the normal 12.5 h duration during one of the periods in which no supplement was fed in order to determine the concentrations of ammonia present in the rumen when supplement was neither fed nor administered intraruminally. This portion of the trial was conducted after the animals had been fed the control diet for 7 days.

During the experiment (March 26 to July 3, 1974), the steers were fed and confined in an outdoor pen with an open-front shed for shelter. The collections of rumen fluid were completed indoors (approximate temperature of 20C).

Results and discussion

Basal concentrations of ammonia in rumen fluid

Ruminal ammonia concentrations in the steers to

which supplemental protein was neither fed immediately prior to the test nor administered on the test day were very low (0.4 - 1.3 mM) throughout the test period. The higher values occurred during the last 3 h (Table 20; Figure 1).

Other researchers have reported relatively low ruminal ammonia concentrations. Fonnesbeck *et al.* (1970) fed a diet consisting entirely of "meadow hay" containing 1.25% N to sheep and found that ruminal ammonia concentrations varied from a maximum of 6.9 mM 3 h after feeding to a minimum of 1.6 mM 12 h after feeding. Annison *et al.* (1954) reported that ruminal ammonia concentrations in sheep fed flaked maize as the main source of protein varied from 0 - 7.5 mg NH_3-N/100 ml (0 - 5.4 mM) during the period of 1 to 6 h after feeding.

The effect of time on change of ruminal ammonia concentrations

Soybean meal:

Ammonia accumulated relatively slowly after the administration of soybean meal on day 1. The maximal increase (7.6 mM) occurred at 6 h while the increase at 12.5 h was 5.6 mM (Table 21; Figure 2). On day 8, the changes in ruminal ammonia concentrations were apparently higher

Table 20. Ammonia concentrations in rumen fluid from four steers fed the control diet for 7 days without the administration of supplement on the test day

Time after start of trial (h)	Mean ammonia concentration in rumen fluid (m mole/ℓ) ± SE[1]
0	0.5 ± 0.03
0.5	0.4 ± 0.06
1.0	0.4 ± 0.06
1.5	0.4 ± 0.14
2.0	0.4 ± 0.09
2.5	0.4 ± 0.06
3.0	0.5 ± 0.09
3.5	0.6 ± 0.11
4.0	0.6 ± 0.15
4.5	0.6 ± 0.11
5.0	0.7 ± 0.13
5.5	0.7 ± 0.11
6.0	0.7 ± 0.20
6.5	0.8 ± 0.22
7.5	0.8 ± 0.21
8.5	0.6 ± 0.25
9.5	0.7 ± 0.24
10.5	0.9 ± 0.30
11.5	1.0 ± 0.24
12.5	1.3 ± 0.21

[1] Standard error of the mean.

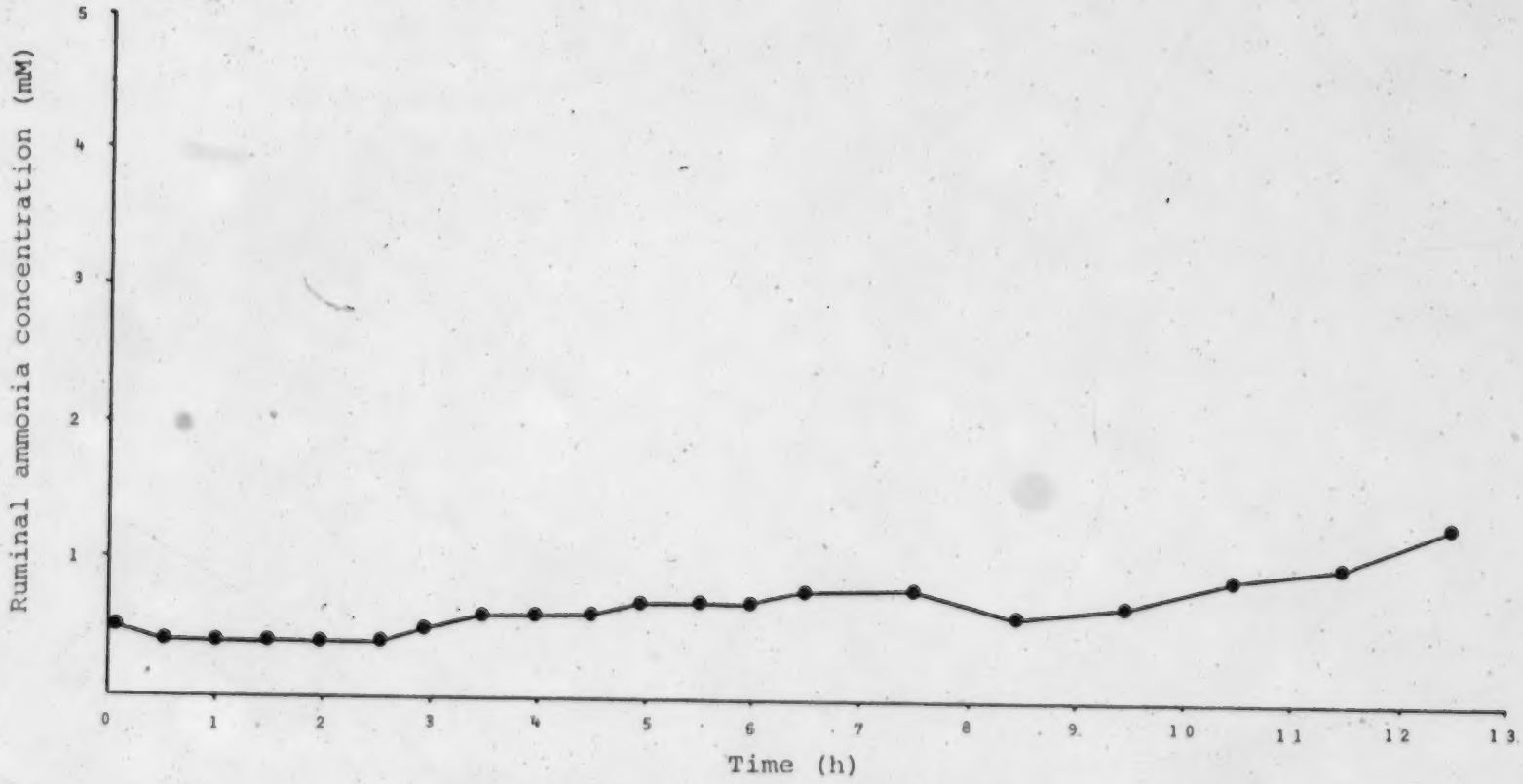

Figure 1. Mean ruminal ammonia concentrations of steers which received neither feed nor water for 12 h prior to sampling

Table 21. Mean changes in ammonia concentrations in rumen fluid from four steers fed a diet containing soybean meal over a 15-day interval and administered soybean meal (773 g*) on days 1, 8 and 15§

Time after start of trial (h)	Mean ammonia difference in rumen fluid (m mole/ℓ) ± SE[1]		
	Day 1	Day 8	Day 15
0.5	-0.3 ± 0.5	3.8 ± 1.2	1.2 ± 0.9
1.0	0.9 ± 0.3	4.7 ± 1.4	2.6 ± 1.6
1.5	1.9 ± 0.8	8.8 ± 1.6	3.8 ± 1.3
2.0	2.6 ± 0.6	9.0 ± 1.9	5.9 ± 2.1
2.5	2.6 ± 0.6	10.0 ± 1.4	4.6 ± 1.4
3.0	3.8 ± 0.8	11.4 ± 1.8	5.2 ± 2.7
3.5	5.1 ± 1.3	15.8 ± 3.0	4.7 ± 1.9
4.0	6.1 ± 1.5	15.3 ± 2.8	5.3 ± 2.0
4.5	7.0 ± 1.9	16.0 ± 2.2	7.8 ± 1.8
5.0	6.8 ± 1.7	17.9 ± 1.5	7.3 ± 2.0
5.5	7.3 ± 2.0	15.8 ± 3.0	7.2 ± 1.1
6.0	7.6 ± 2.1	13.8 ± 1.6	7.7 ± 2.3
6.5	7.4 ± 1.9	11.3 ± 2.6	10.5 ± 2.1
7.5	7.5 ± 2.0	12.6 ± 1.7	11.0 ± 2.5
8.5	5.6 ± 1.2	11.6 ± 1.8	8.6 ± 2.6
9.5	7.1 ± 2.1	12.0 ± 1.6	8.4 ± 2.2
10.5	6.4 ± 1.7	11.2 ± 0.9	10.2 ± 2.5
11.5	6.5 ± 2.5	10.4 ± 1.3	11.5 ± 3.2
12.5	5.6 ± 1.5	9.8 ± 0.8	6.2 ± 3.0

* Supplied 60 g of N.

[1] Standard error of the mean.

§ Mean initial concentration: 6.4 ± 1.2.

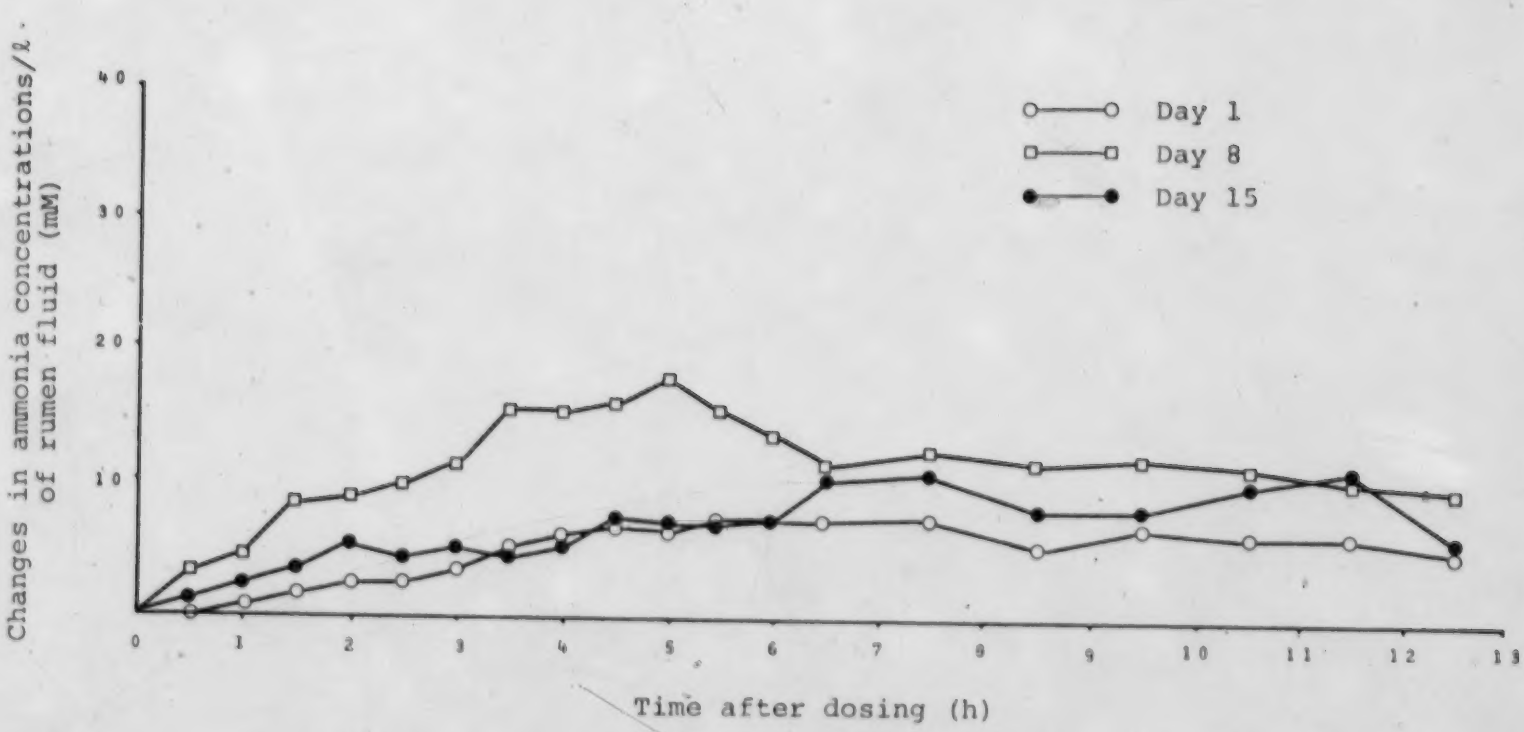

Figure 2. Mean changes in ruminal ammonia concentrations of four steers dosed with 773 g of soybean meal (60 g of N) on days 1, 8 and 15 of adaptation to 72 g of N/steer/day as soybean meal

than those on day 1. The maximal increase (17.9 mM), however, occurred at 5.0 h. The changes in concentration on day 15 were apparently higher than those on day 1 during the periods of 0 to 3 h and 6 to 12.5 h (Figure 2).

While there appear to be differences in changes of ruminal ammonia concentration between days 1 and 8, the fact that the changes on day 15 were intermediate between those of day 1 and day 8 and, in some cases, very similar to those of day 1 makes any suggestion of adaptation to soybean meal difficult to substantiate.

Urea:

While soybean meal, BBGU and BBMGU were administered at rates sufficient to supply 60 g of N per head on days 1, 8 and 15, urea was administered in amounts sufficient to supply 30 g of N on each test day, due to the danger of toxicity at the higher level.

The maximal increase of ruminal ammonia concentration on day 1 (33.4 mM) occurred at 2.5 h after dosing with urea. The changes in concentration then declined to a minimum of 7.6 mM at 12.5 h (Table 22; Figure 3). On day 8, the maximal change in concentration (52.6 mM) occurred at 1 h and the minimum (3.9 mM) at 12.5 h. The maximal increase on day 15 (44.1 mM) occurred at 2 h. The changes in concentration during the period of 4.5 to 11.5 h on day

Table 22. Mean changes in ammonia concentrations in rumen fluid from four steers fed a diet containing urea over a 15-day interval and administered urea (67 g*) on days 1, 8 and 15§

Time after start of trial (h)	Mean ammonia difference in rumen fluid (m mole/ℓ) ± SE[1]		
	Day 1	Day 8†	Day 15
0.5	16.4 ± 9.7	24.5 ± 1.7	21.6 ± 2.5
1.0	27.6 ± 8.4	52.6 ± 13.9	32.6 ± 4.8
1.5	29.6 ± 7.6	44.1 ± 9.7	37.0 ± 3.8
2.0	29.5 ± 8.7	42.7 ± 7.5	44.1 ± 6.0
2.5	33.4 ± 4.4	38.6 ± 2.6	39.4 ± 5.5
3.0	30.4 ± 5.7	36.3 ± 3.5	39.0 ± 11.2
3.5	25.2 ± 4.6	34.6 ± 4.1	26.7 ± 6.1
4.0	24.7 ± 7.2	30.2 ± 1.5	22.6 ± 3.8
4.5	28.4 ± 4.3	29.8 ± 3.5	27.0 ± 7.6
5.0	24.4 ± 2.7	26.9 ± 4.0	19.6 ± 1.7
5.5	24.4 ± 5.1	22.7 ± 2.7	14.0 ± 3.6
6.0	21.9 ± 3.3	21.6 ± 4.5	13.1 ± 3.1
6.5	21.4 ± 1.5	19.4 ± 3.1	9.5 ± 3.1
7.5	16.8 ± 1.8	17.1 ± 1.3	8.3 ± 3.2
8.5	14.2 ± 2.5	14.1 ± 1.4	3.9 ± 3.2
9.5	11.0 ± 2.3	12.2 ± 1.6	4.8 ± 2.1
10.5	9.1 ± 2.6	8.5 ± 3.3	2.6 ± 1.7
11.5	7.9 ± 2.1	6.7 ± 3.6	3.1 ± 2.1
12.5	7.6 ± 2.2	3.9 ± 3.3	4.8 ± 1.7

* Supplied 30 g of N.

[1] Standard error of the mean.

† Due to an error in administration the data for one animal were not used. The data for day 8, therefore, are based on data from three animals.

§ Mean initial concentration: 6.2 ± 1.0.

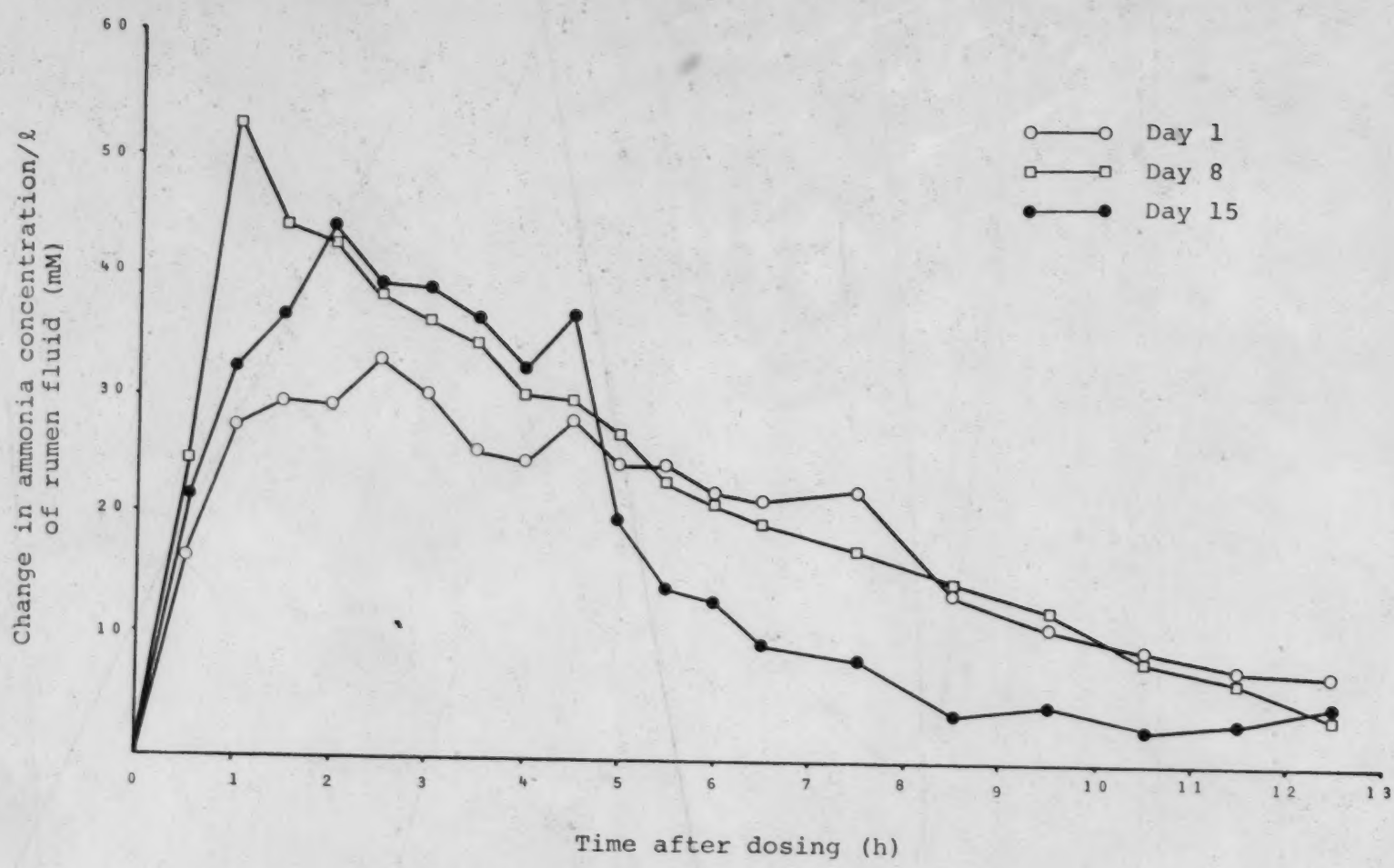

Figure 3. Mean changes in ruminal ammonia concentrations of four steers dosed with 67 g of urea (30 g of N) on days 1, 8 and 15 of adaptation to 59 g of N/steer/day as urea

15 were apparently lower than those at comparable times on days 1 and 8.

Oltjen (1969), in a review paper, stated that feeding steers purified diets which contain urea as the primary N source usually results in maximal ruminal ammonia concentrations of 35-55 mg NH_3-N/100 ml (25.0 - 39.3 mM) at 1 to 1.5 h after feeding.

The rates of ammonia accumulation from urea on days 1, 8 and 15 were similar to each other. Thus, there was no evidence to suggest that adaptation to urea occurred in this experiment.

BBGU:

On day 1, the increase in ruminal ammonia concentration reached a maximum (29.0 mM) at 3 h, declined rapidly to 16.1 mM at 4 h and then decreased to a minimum of 8.2 mM over the next 8 h (Table 23; Figure 4). The maximal increase on day 8 was 35.4 mM at 4 h after the test dose was administered. The mean increases in ruminal ammonia concentration on day 8 were higher, with one exception, than those on day 1. The apparent differences in concentration were most pronounced during the period of 3 to 12.5 h. The maximal increase on day 15 was 33.4 mM at 3.5 h. The increases on day 15 were similar to those on day 8 but were somewhat lower during the period of 3 to

Table 23. Mean changes in ammonia concentrations in rumen fluid from four steers fed a diet containing BBGU over a 15-day interval and administered BBGU (751 g*) on days 1, 8 and 15§

Time after start of trial (h)	Mean ammonia difference in rumen fluid (m mole/ℓ) ± SE[1]		
	Day 1	Day 8	Day 15
0.5	18.4 ± 6.4	21.4 ± 8.1	13.5 ± 5.8
1.0	25.0 ± 4.7	24.9 ± 7.0	27.5 ± 1.3
1.5	24.2 ± 6.4	27.8 ± 5.3	25.8 ± 3.4
2.0	27.1 ± 3.7	30.5 ± 4.6	32.2 ± 4.4
2.5	20.0 ± 4.0	32.8 ± 7.7	29.6 ± 6.6
3.0	29.0 ± 6.6	30.5 ± 4.0	27.3 ± 3.9
3.5	22.0 ± 6.6	30.5 ± 4.3	33.4 ± 2.9
4.0	16.1 ± 3.9	33.9 ± 5.8	31.5 ± 4.2
4.5	17.3 ± 4.3	35.4 ± 7.7	28.8 ± 1.2
5.0	15.7 ± 3.3	32.3 ± 7.1	26.8 ± 5.4
5.5	14.4 ± 2.9	28.6 ± 6.7	25.4 ± 3.3
6.0	13.4 ± 2.5	29.8 ± 7.0	25.4 ± 3.7
6.5	13.6 ± 2.7	27.7 ± 5.6	21.9 ± 2.5
7.5	11.2 ± 2.2	28.5 ± 5.0	23.6 ± 2.1
8.5	11.2 ± 3.0	24.7 ± 3.2	20.0 ± 2.0
9.5	10.9 ± 3.1	23.2 ± 4.1	17.3 ± 3.8
10.5	9.3 ± 3.9	17.8 ± 4.0	12.8 ± 2.9
11.5	8.2 ± 3.5	16.3 ± 3.4	11.6 ± 2.1
12.5	9.1 ± 4.1	13.5 ± 2.0	11.2 ± 1.9

* Supplied 60 g of N.

[1] Standard error of the mean.

§ Mean initial concentration: 5.1 ± 0.7.

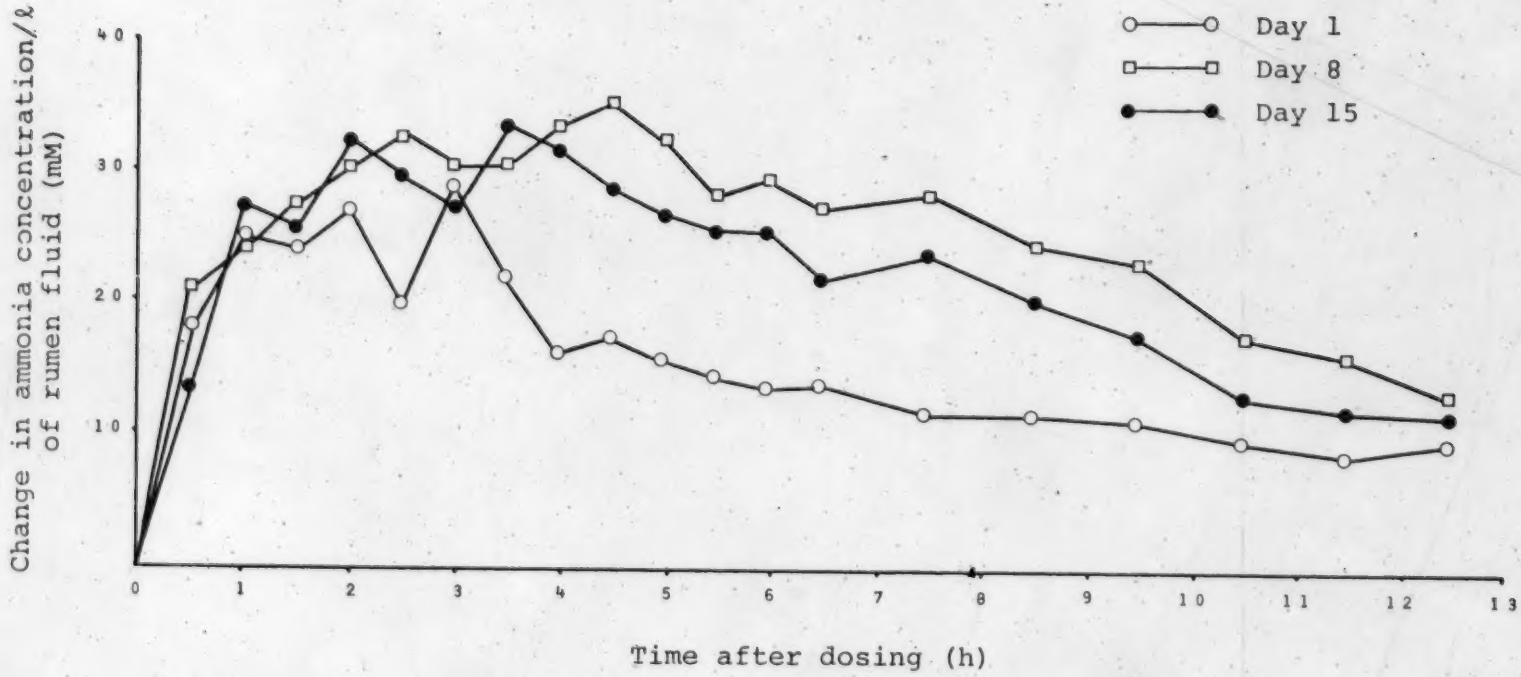

Figure 4. Mean changes in ruminal ammonia concentrations of four steers dosed with 751 g of BBGU (60 g of N) on days 1, 8 and 15 of adaptation to 69 g of N/steer/day as BBGU

12.5 h.

Some adaptation would appear to have occurred during the period of day 1 to day 8. While the increases in ruminal ammonia concentrations during the period 0 to 3.5 h were similar for all collection dates, there were apparent differences between day 1 and day 8 and between day 1 and day 15 during the period of 3 to 12.5 h. The differences during the latter period suggest that the ability of the rumen microorganisms to degrade BBGU was enhanced during the first 7 days it was fed.

These results are compatible with those of Milligan *et al.* (1972) who found apparent adaptation to glucosyl-ureide during a period of 7 days. Atwal, Young and Milligan (1971) obtained similar results with mixed amides.

BBMGU:

The administration of test doses of BBMGU did not increase ruminal ammonia concentrations as much as did the administration of soybean meal, urea or BBGU.

On day 1, ruminal ammonia concentrations increased very slowly to a maximum of 3.5 mM at 11.5 h (Table 24; Figure 5). The concentrations on day 8 were higher than those on day 1 during the period of 0 to 9.5 h and lower for the remainder of the test period. The changes

Table 24. Mean changes in ammonia concentrations in rumen fluid from four steers fed a diet containing BBMGU over a 15-day interval and administered BBMGU (765 g*) on days 1, 8 and 15§

Time after start of trial (h)	Mean ammonia difference in rumen fluid (m mole/ℓ) ± SE[1]		
	Day 1	Day 8	Day 15
0.5	1.0 ± 0.3	1.4 ± 0.6	1.3 ± 0.8
1.0	1.1 ± 0.3	3.4 ± 2.2	3.3 ± 2.4
1.5	1.3 ± 0.3	6.7 ± 2.2	4.0 ± 1.7
2.0	1.2 ± 0.4	6.8 ± 3.0	5.4 ± 1.8
2.5	1.6 ± 0.5	10.8 ± 2.9	3.7 ± 1.5
3.0	1.9 ± 0.5	9.6 ± 4.0	4.6 ± 1.6
3.5	2.1 ± 0.6	12.0 ± 2.9	4.9 ± 1.5
4.0	2.2 ± 0.6	9.7 ± 3.5	5.8 ± 1.9
4.5	2.4 ± 0.3	9.3 ± 3.5	4.6 ± 1.6
5.0	2.4 ± 0.4	7.4 ± 2.6	2.4 ± 1.5
5.5	2.8 ± 0.3	8.8 ± 2.8	1.2 ± 0.9
6.0	2.6 ± 0.2	6.1 ± 1.8	1.0 ± 1.2
6.5	3.6 ± 1.5	6.4 ± 1.8	0.3 ± 1.3
7.5	2.6 ± 0.2	5.0 ± 1.7	0.7 ± 1.0
8.5	3.2 ± 0.7	5.0 ± 1.7	0.2 ± 0.8
9.5	2.8 ± 0.3	3.2 ± 1.3	-0.3 ± 0.7
10.5	2.9 ± 0.4	2.0 ± 0.9	-0.2 ± 0.8
11.5	3.5 ± 1.0	1.7 ± 0.6	-0.3 ± 1.2
12.5	2.4 ± 0.3	1.5 ± 0.6	-1.1 ± 1.1

* Supplied 60 g of N.

[1] Standard error of the mean.

§ Mean initial concentration: 4.6 ± 0.4.

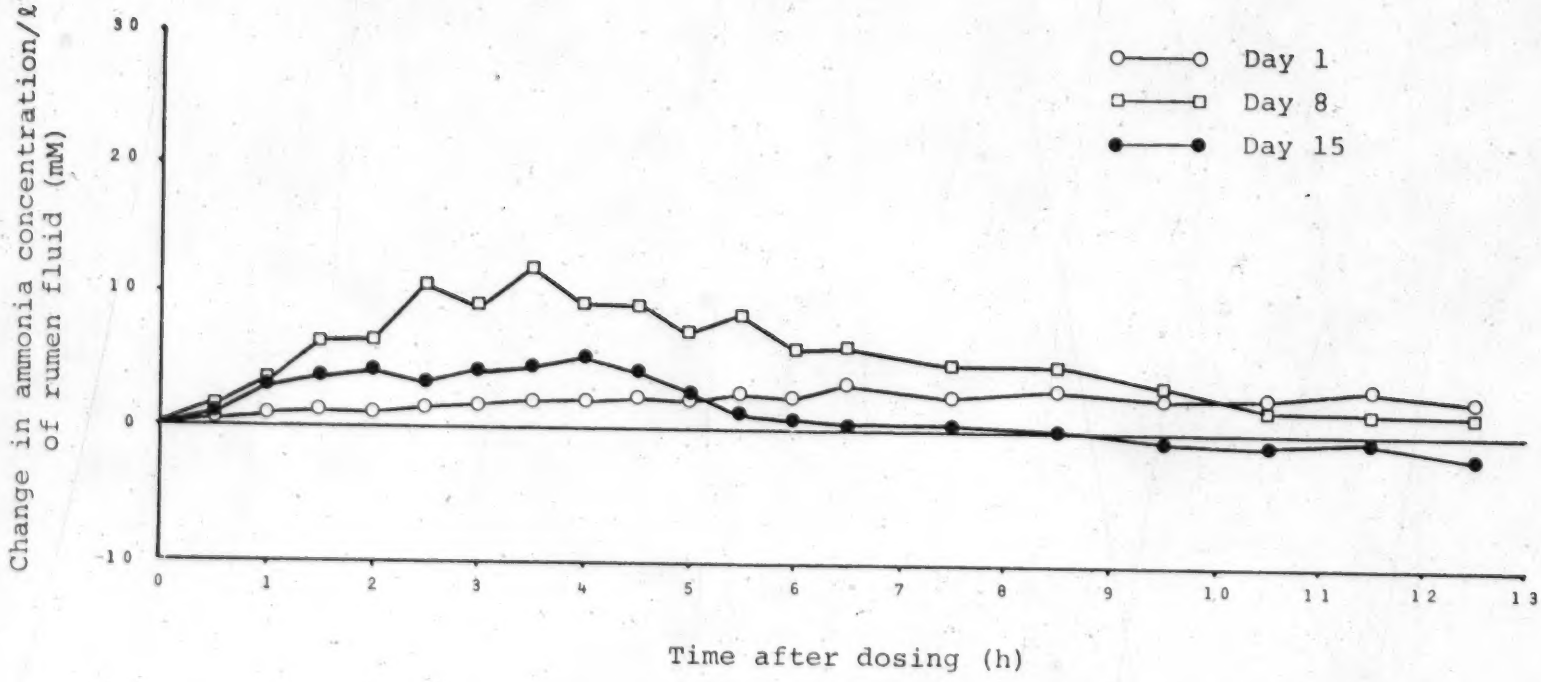

Figure 5. Mean changes in ruminal ammonia concentrations of four steers dosed with 765 g of BBMGU (60 g of N) on days 1, 8 and 15 of adaptation to 78 g of N/steer/day as BBMGU

in ruminal ammonia concentration on day 15 during the period of 0 to 5 h were higher than those on day 1 and lower than those on day 8. However, during the period of 5 to 12.5 h, the increases in concentration on day 15 were lower than those on day 1 and day 8.

There was no evidence of adaptation to BBMGU in this experiment. However, Lin and Mathison (unpublished data) observed that the maximal rate of ammonia accumulation was achieved within 7 days when a test mixture of 10% MGU and 90% GU was administered while 7 to 14 days were apparently required to achieve adaptation when a mixture of 30% MGU and 70% GU was used.

The effects of nitrogen sources on changes in ruminal ammonia concentrations

Day 1:

The administration of urea in an amount sufficient to provide 30 g of N resulted in a maximal increase in ruminal ammonia concentration of 33.4 mM at 2.5 h. The maximal increases caused by the administration of soybean meal (7.6 mM), BBGU (29.0 mM) and BBMGU (3.5 mM) were lower than that resulting from the administration of urea even though each of these supplements was administered in an amount sufficient to supply 60 g of N (Figure 6). The

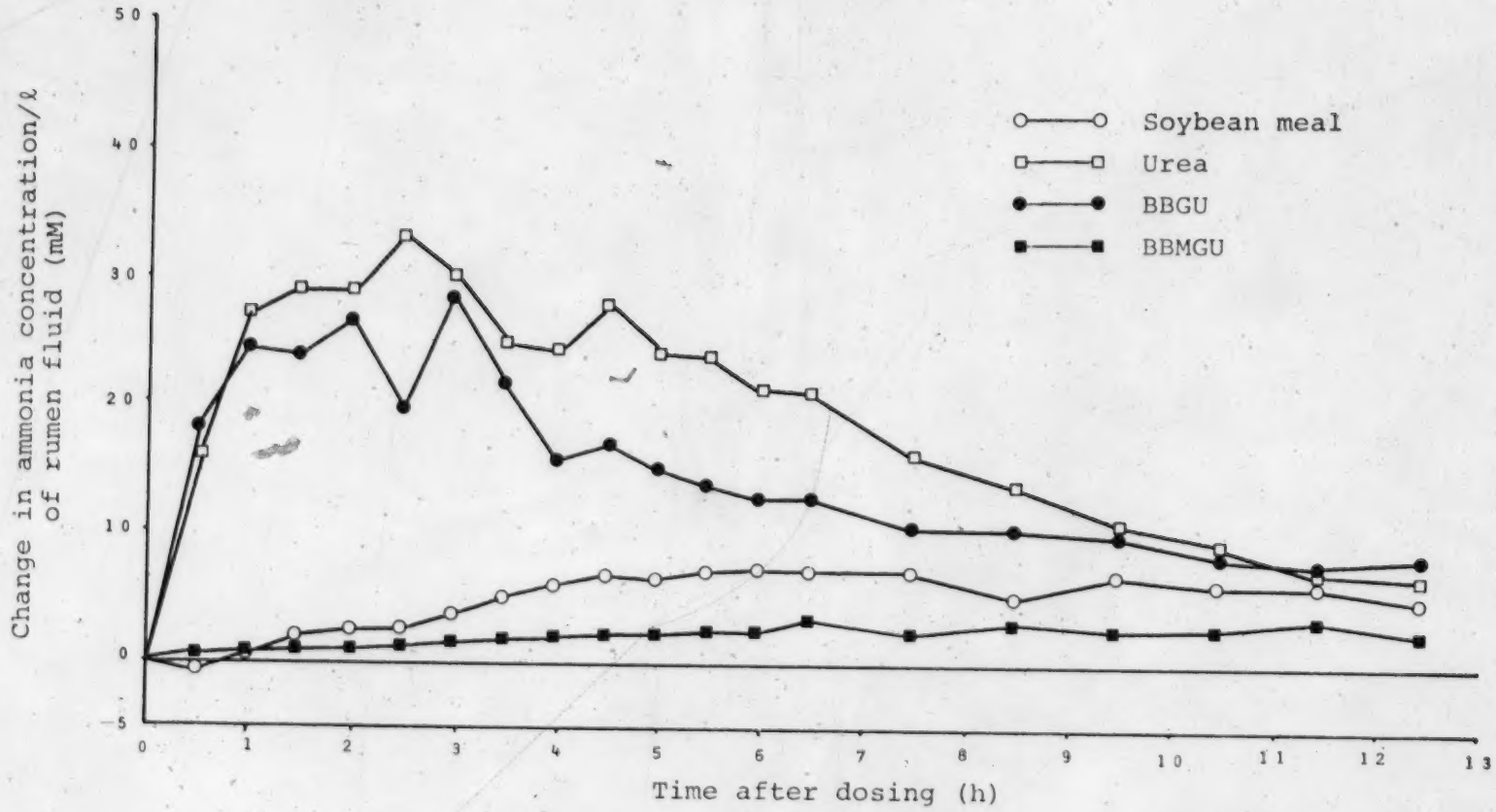

Figure 6. Mean changes in ruminal ammonia concentrations of four steers dosed with 773 g of soybean meal, 67 g of urea, 751 g of BBGU or 765 g of BBMGU on day 1 of adaptation to soybean meal, urea, BBGU and BBMGU, respectively

maximal increases occurred comparatively early in the test period when urea and BBGU were administered (2.5 h and 3.0 h, respectively) while the maximal increases resulting from dosage with soybean meal and BBMGU occurred comparatively late in the test period (6 h and 11.5 h, respectively).

Day 8:

The maximal increase in ruminal ammonia concentration resulting from the administration of urea was 52.6 mM at 1 h, while the maximal increases for the animals fed BBGU, soybean meal and BBMGU were 35.4 (4.5 h), 17.9 (5.0 h) and 12.0 (3.5 h), respectively (Figure 7). The increases resulting from the administration of urea were larger than those of BBGU, soybean meal and BBMGU during the period 0 to 3.5 h but were exceeded by those of BBGU during the period 3.5 to 12.5 h. The changes in concentration in animals to which soybean meal was administered also exceeded those of the urea group during the period 9.5 to 12.5 h. Urea, therefore, appeared to have been degraded more rapidly than the other N supplements, resulting in comparatively large increases in rumen ammonia during the first part of the test period and small increases during the latter part of the test period (Figure 7).

The changes in ruminal ammonia concentrations in

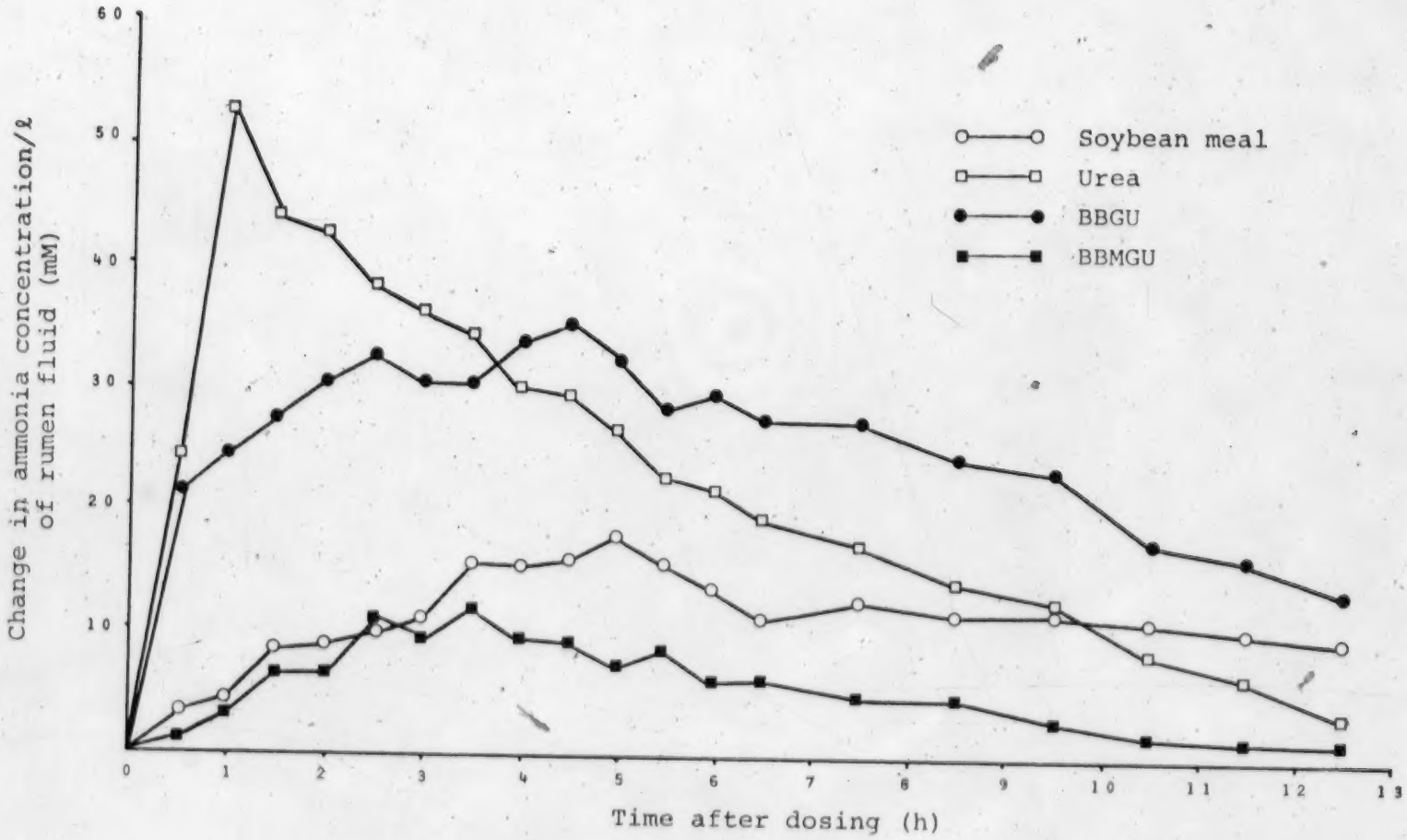

Figure 7. Mean changes in ruminal ammonia concentrations of four steers dosed with 773 g of soybean meal, 67 g of urea, 751 g of BBGU or 765 g of BBMGU on day 8 of adaptation to soybean meal, urea, BBGU and BBMGU, respectively

the soybean meal and BBGU groups reached maximum levels 3.5 to 4 h later than those of the urea group and did not decline as quickly after the maximum levels had been reached. The changes in ruminal ammonia levels in the BBGU group reached a maximum at 3.5 h and then declined to a minimum level (1.5 mM) which was considerably lower than those of the other groups.

Day 15:

The change in ruminal ammonia concentration resulting from the administration of urea (30 g of N) reached a maximum of 44.1 mM at 2 h, then declined rapidly to a minimum of 2.6 mM at 10.5 h. BBGU (maximum change, 33.4 mM at 3.5 h; minimum change, 11.2 mM at 12.5 h), when administered in an amount sufficient to supply 60 g of N, did not increase ruminal ammonia concentrations as much as the urea dose during the period 0 to 3 h but caused higher changes during the period 3 to 12.5 h (Figure 8).

Soybean meal, like BBGU and BBMGU, was administered in an amount sufficient to supply 60 g of N. The maximal change in ruminal ammonia concentration (11.5 mM) was reached at 11.5 h. The concentrations during the period 10.5 to 12.5 h were similar to those resulting from dosage with BBGU and slightly higher than those attributable to urea and BBMGU.

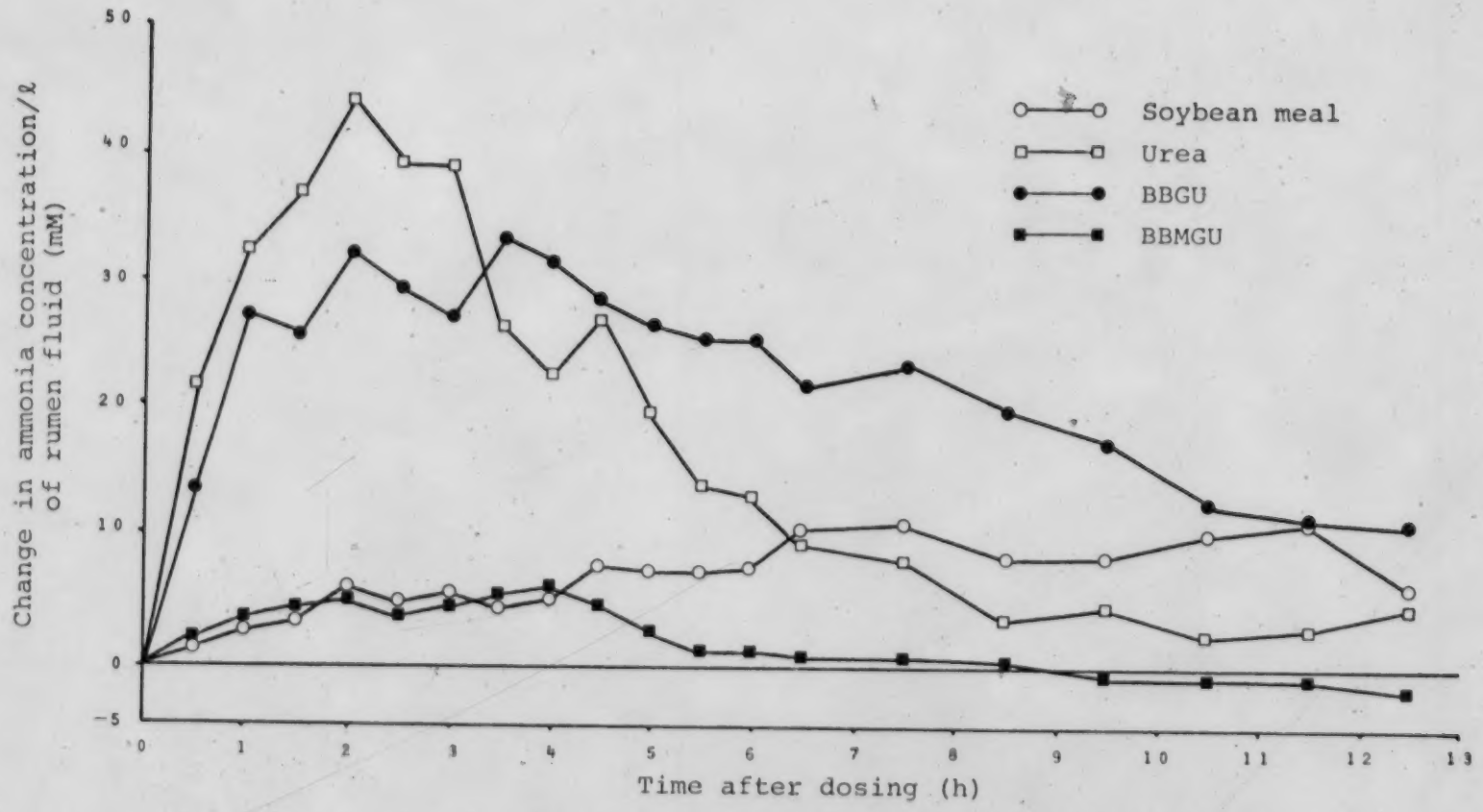

Figure 8. Mean changes in ruminal ammonia concentrations of four steers dosed with 773 g of soybean meal, 67 g of urea, 751 g of BBGU or 765 g of BBMGU on day 15 of adaptation to soybean meal, urea, BBGU and BBMGU, respectively

BBMGU caused a maximal increase (5.4 mM) at 2 h. The values declined after 2 h and, during the period of 9.5 to 12.5 h, were negative. The minimum change observed was -1.1 mM at 12.5 h.

Davis and Stallcup (1967) fed steers diets containing urea, soybean meal and urea plus soybean meal. The N supplements were fed once daily in amounts calculated to supply 0.79 kg of digestible protein. The amounts of soybean meal and urea fed supplied 139 g and 128 g of N, respectively. Ruminal ammonia concentrations were measured at 0, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after feeding during the third week of adaptation. The maximal increase in ruminal ammonia concentration in the animals fed soybean meal was 11 mg NH_3-N/100 ml (7.8 mM) at 3 h while the minimum increase was 5 mg NH_3-N/100 ml (3.6 mM) at 10 h. The changes in ruminal ammonia concentrations resulting from the feeding of urea reached a maximum of 43 mg NH_3-N/100 ml (30.7 mM) at 2.5 h and a minimum of 31 mg NH_3-N/100 ml (22.1 mM) at 12 h.

The experiment conducted by Davis and Stallcup (1967) differs from the experiment reported here in amounts of N supplements used and the method of administration. Nevertheless, both experiments show the rapid increase and decline in ruminal ammonia resulting from the degradation of urea in the rumen and the comparatively small but con-

sistent changes in rumen ammonia attributable to the degradation of soybean meal.

The determination of ruminal ammonia concentrations during a particular interval does not measure the amount of ammonia which evolved from a dietary N source nor does it measure the rate at which ammonia was produced. Ruminal ammonia concentration is influenced by many factors including the rate at which ammonia is produced in the rumen, the rate at which ammonia is utilized by rumen microorganisms and the amount of ammonia absorbed by the animal (Chalmers, 1961).

The procedure used in this experiment, whereby test doses of specific products are administered at three intervals after the commencement of feeding the products, does allow, however, for the rapid evaluation of ammonia accumulation patterns and rates as well as the time required for adaptation to each product (Atwal *et al.*, 1971). The technique also allows for the assessment of potential toxicity through the comparison of new NPN sources with urea, in terms of rates of ammonia accumulation.

In this experiment, adaptation was apparent within seven days after BBGU was first fed. There was not sufficient evidence to suggest that adaptation to soybean meal, urea or BBMGU occurred. The rates of change of ruminal ammonia concentrations on day 15 were similar or

somewhat lower than those found on day 8 for each of the N sources tested. Milligan (1972) and Atwal *et al.* (1971) obtained similar results with GU and mixed amides.

Urea and BBGU increased ruminal ammonia concentrations more rapidly than soybean meal and BBMGU, both of which provided comparatively consistent, but lower, rates of increase in ammonia concentrations during the test period (Figures 6, 7 and 8). The fact that the BBGU preparation used in this trial contained 2.83% urea may explain the relatively rapid increase in rumen ammonia during the first 3 h after it was administered. Neither BBGU nor BBMGU, when administered in amounts sufficient to supply 60 g of N, increased rumen ammonia concentrations nearly as rapidly as 67 g of urea (30 g of N). BBGU and BBMGU, therefore, would likely be much less toxic per unit of N than urea. BBGU provided for less rapid increases in ruminal ammonia during the first 3 h of the 12.5 h period and higher increases during the latter part of the 12.5 h period on days 8 and 15 than those obtained from urea. Therefore, BBGU would be expected to be utilized more efficiently than urea by rumen microorganisms, where the diets used are fermented slowly. BBMGU, however, appeared to be degraded very slowly or perhaps incompletely.

PART V

INVESTIGATION OF THE POTENTIAL TOXICITY OF BBGU

Introduction

Urea is hydrolyzed rapidly in the rumen to ammonia and carbon dioxide (Huhtanen and Gall, 1955). Urea, when consumed in excessive amounts, may cause ammonia toxicity, the symptoms of which include laboured breathing, ataxia, excessive salivation, cellular changes in nervous tissue and death (Chalupa, 1968; Gibson *et al.*, 1974; Word *et al.*, 1969). Consequently, care must be taken in the use of urea in diets for ruminants.

Some NPN supplements are apparently non-toxic when fed to ruminants. Biuret can be used in diets for ruminants without danger of toxicity due to the slow rate of ammonia accumulation in the rumen when it is fed (Fonnesbeck *et al.*, 1975).

The objective of this experiment was to determine if the use of BBGU at various levels in a diet would cause toxicity or any detectable physiological abnormalities in lambs.

Materials and methods

Twenty-four crossbred wether lambs were purchased

locally for use in the experiment. The lambs were fed a diet consisting of 80% barley grain and 20% alfalfa hay (by weight) during a 14-day adjustment period and then allocated randomly to five treatment groups. Groups 1, 2, 3 and 5 each consisted of five animals, while group 4 contained four animals. The animals were housed indoors in wooden pens (average temperature of 21C). Water was supplied by automatic waterers. Each group was fed a different concentrate mixture *ad libitum*. The concentrate mixtures fed to groups 1, 2, 3, 4 and 5 contained on a weight 'as fed' basis, 10% BBGU, 20% BBGU, 50% BBGU, 10% soybean meal and 99.85% BBGU (Table 25). Alfalfa pellets (12.7% crude protein on a DM basis) were fed to all animals at the rate of 250 g per head per day. A mineral mixture containing, by weight, 25% limestone, 25% salt and 50% calcium phosphate was fed on an *ad libitum* basis.

The lambs (average initial body weight of 31.0 kg) were weighed every 14 days. At the end of a 63-day trial period, two sheep from each group were slaughtered. *Post mortem* examination of the animals was conducted by Dr. B.E. Beck and staff of the Veterinary Services Division, Alberta Agriculture, Edmonton.

On day 63 of the trial, 15 ml blood samples were collected from each of the animals by jugular vein puncture

Table 25. Formulation of concentrate mixtures fed to sheep (*ad libitum*) in toxicity study†

	Group 1	Group 2	Group 3	Group 4	Group 5
Ingredients (%)					
BBGU	10.0	20.0	50.0	-	99.85
Limestone	0.66	0.92	1.67	0.45	-
Salt (cobalt-iodized)	0.50	0.50	0.50	0.50	-
Sulphur	0.10	0.20	0.50	-	-
Vitamin premix*	0.15	0.15	0.15	0.15	0.15
Steam-rolled barley§	88.59	78.23	47.18	89.9	-
Soybean meal (48.5% protein)	-	-	-	10.0	-

† A mineral mixture containing 25% limestone, 25% salt (cobalt-iodized) and 50% calcium phosphate (18.5% Ca and 20.5% P) was fed *ad libitum*.

* To supply 1350, 223 and 0.1 IU of vitamins A, D and E, respectively, per kg of concentrate mixture.

§ Ground barley was substituted for steam-rolled barley in Group 3 due to separation and selection problems.

using 20-gauge needles and vacuum tubes containing heparin. The samples were placed immediately in ice, transported to the laboratory and centrifuged at 671 x g for 25 min in accordance with standard procedures. The plasma was withdrawn and stored at -25C for subsequent analyses. Analyses for PUN were carried out using the method of Fawcett and Scott (1960) as adapted for use with the Technicon Auto-Analyzer (Agricultural Soil and Feed Testing Laboratory Manual, 1975).

The data were analyzed by means of analysis of variance, using a program (AOV5) available through The University of Alberta Computing Centre. Means were compared through the use of Duncan's multiple range test (Steel and Torrie, 1960). A probability of 0.05 was used, in all cases, as the point of significance.

Results and discussion

The mean daily gains, daily feed intakes and feed:gain ratios for the five treatment groups are listed in Table 26.

The mean amounts of total DM consumed for the groups fed the concentrate mixtures containing, on a weight 'as fed' basis, 10% BBGU, 20% BBGU and 10% soybean meal were similar (1.94, 1.83 and 1.89 kg per head per day, respectively). The sheep fed the two highest levels of

Table 26. Average daily gains, feed intakes and feed:gain ratios of lambs fed concentrate mixtures containing four concentrations of BBGU and one concentration of soybean meal

Supplement (%) included in concentrate mixture	Average daily gain (kg)	Average daily feed intake (kg)	Feed:gain ratio	BBGU in total diet (%)
BBGU (10%)	0.37*a*	1.94	5.24	8.7
BBGU (20%)	0.34*ab*	1.83	5.38	17.3
BBGU (50%)	0.23*ab*	1.61	7.00	42.2
Soybean meal (10%)	0.41*a*	1.89	4.61	-
BBGU (99.85%)	-0.03*b*	0.60	-	58.3
SE[1]	0.03	0.25	0.51	

a, *b* Means followed by different letters in the same row are significantly different ($P < 0.05$).

[1] Standard error of the mean.

BBGU consumed less DM than the other groups; the average daily consumption per head of the group fed the 50% BBGU mixture was 1.61 kg while that of the group fed the 99.85% mixture was 0.60 kg. The animals fed the mixture containing 50% BBGU initially tended to eat the steam-rolled barley in the mixture and leave the BBGU. The substitution of ground barley for rolled barley in this diet eliminated this sorting and selection.

One animal in the 20% BBGU group was withdrawn from the experiment after 52 days, due to blockage of the urethra. The problem was considered to be unrelated to the experimental diet.

There were no statistical differences in rate of gain between the 10% BBGU, 20% BBGU, 50% BBGU and 10% soybean meal groups. The daily change in weight (-0.03 kg) for the animals of the 99.85% BBGU group was significantly lower than the average daily gains of the 10% BBGU and 10% soybean meal groups but not significantly different from those of the 20% BBGU and 50% BBGU groups. The feed:gain ratios for the 10% BBGU, 20% BBGU, 50% BBGU and 10% soybean meal groups were 5.24, 5.38, 7.00 and 4.61, respectively. Thus, the animals fed the 50% BBGU diet tended to consume less DM and gain less weight per day than the animals fed the 10% BBGU, 20% BBGU and 10% soybean meal diets.

Mean daily N intakes and PUN concentrations on

day 63 are listed in Table 27. The PUN concentrations tend to reflect mean N intakes, except in the case of the 99.85% BBGU group. The blood samples for PUN determination, however, were taken on the final day of the trial at which time the mean weight of this group was 29.5 kg as compared to 53.8 kg, 53.3 kg, 44.9 kg and 31.3 kg for the groups fed 10% BBGU, 20% BBGU, 50% BBGU and 10% soybean meal, respectively.

The sheep in the 50% BBGU and 99.85% BBGU groups pulled and ate wool from their pen-mates and from themselves. The animals in the 99.85% BBGU group were very thin, emaciated, weak and somewhat uncoordinated. *Post mortem* examination did not reveal, however, any gross lesions or abnormalities in this group or in the other four groups. There were mild infestations of roundworms (*Nematodirus* sp.) in all of the sheep examined. The infestations, although sub-clinical, were more severe in the groups fed 50% BBGU and 99.85% BBGU. There were no visible lesions in the adrenal glands, spinal cord, thymus, muscle or kidneys (Beck, 1974).

There were areas of demyelination in the brain of one of the sheep fed the 99.85% BBGU mixture. These areas occurred with greatest intensity in the mid-brain, in the dorsal pontine tracts and in the cerebellar peduncles. In association with this demyelination in the mid-brain,

Table 27. Mean daily nitrogen intakes and mean plasma urea nitrogen concentrations for lambs fed four concentrations of BBGU and one concentration of soybean meal

Supplement (%) in concentrate mixture	Nitrogen intake/day (g)	Plasma urea nitrogen (mg/100 ml)
BBGU (10%)	43	19.7*ab*
BBGU (20%)	51	26.7*b*
BBGU (50%)	72	44.7*c*
Soybean meal (10%)	41	16.8*a*
BBGU (99.85%)	34	45.4*c*
Standard error of the mean	14.6	2.81

a, *b*, *c* Means followed by different letters are significantly different ($P < 0.05$).

there was axon swelling and degeneration (Figures 9 and 10). The other sheep fed the 99.85% BBGU mixture showed the same lesions but "to a very minimal degree" (Beck, 1974).

Lesions of demyelination also were found in the brains of the two sheep fed the 50% BBGU mixture and of one of the sheep fed the 20% BBGU mixture.

Beck (1974) stated that the severe lesions which were found in the brain of the sheep fed the 99.85% BBGU diet were characteristic of those associated with ammonia toxicity. The lesions found in the other sheep, in his opinion, reflected less severe cases of ammonia toxicity since they were not accompanied by axon degeneration. Beck suggested that the lesions could cause deranged cerebral function and, therefore, might explain the wool consumption, weakness and uncoordination.

The BBGU contained 8.48% N, of which 1.5% was supplied by barley and the remaining 6.98% by urea. The amount of N (g) from NPN consumed per day per kg of mean body weight for the various diets were: 0.28 (10% BBGU), 0.52 (20% BBGU), 1.25 (50% BBGU) and 0.80 (99.85% BBGU) (Table 28). Several researchers have found the toxic dose of urea, in terms of g of N from urea per kg of body weight, is lower than rates of consumption of N from NPN achieved in this experiment. Chalupa (1968) stated that the toxic level of urea given as a drench is 0.44 - 0.67 g (0.07 -

Figure 9. Early form of neuronal degeneration subsequent to demyelination and axon degeneration, showing margination of nuclei and early central chromatolysis in sheep fed concentrate mixture containing 99.85% BBGU.

Figure 10. Chromatolysis in a degenerating neuron in brain of sheep fed concentrate mixture containing 99.85% BBGU; cytoplasm has become homogeneous and eosinophillic with loss of nissel granules.

Table 28. Initial, final and mean body weights, BBGU consumed and consumption of NPN by lambs fed soybean meal and four concentrations of BBGU

Concentrate mixture fed	Body weights (kg)			Mean BBGU consumed per day (g)	Mean N from NPN consumed	
	Initial	Final	Mean during trial		per day (g)	per day per kg mean body weight (g)
BBGU (10%)	30.4	53.8	42.1	168	11.7	0.28
BBGU (20%)	31.3	53.3	42.3	316.6	22.1	0.52
BBGU (50%)	30.6	44.9	37.8	679.4	47.4	1.25
Soybean meal (10%)	31.4	57.7	44.6	-	-	-
BBGU (99.85%)	31.3	29.5	30.4	349.8	24.4	0.80
SE[1]	0.57	2.30				

[1] Standard error of the mean.

0.11 g of N from urea) per kg of body weight. Kromann *et al.* (1971) found that the "median lethal dose" (LD_{50}) of urea, administered directly in the rumen, was 0.285 g (0.046 g of N from urea) per kg of body weight. Coombe *et al.* (1960), however, fed up to 100 g of urea (45 g of N) per head per day to sheep (mean body weight of 40-50 kg) without any apparent toxic effects. They added an urea-molasses mixture to chopped "meadow hay" which was fed on an *ad libitum* basis. When the level of urea fed exceeded 6% of the DM in the diet voluntary intake declined.

Apparently diets containing at least 17.3% BBGU in the total diet may be fed without danger of toxicity (Table 26). The prolonged use of higher concentrations of BBGU in diets may result in brain lesions as a result of a chronic ammonia toxicity. Further work is required to determine, firstly, if these lesions are caused by ammonia, secondly, the dietary levels of BBGU and other NPN sources which may cause the lesions and, thirdly, the effects of such lesions on animal behaviour and performance.

GENERAL DISCUSSION AND CONCLUSIONS

The apparent inconsistency with which supplemental N in ruminant diets provides demonstrable benefits makes the practical assessment of products such as BBGU and BBMGU difficult. Several trials would be required before firm conclusions with respect to their efficacy could be reached. In the growth trials reported, MGU, when fed as part of a liquid supplement, depressed live-weight gain (Part I) while GU had no effect on daily gain when fed as part of a liquid supplement (Part I) but was as effective as soybean meal in supplying supplemental N, when fed in the form of BBGU (Part II). Perhaps further research with respect to N requirements of ruminants, the results of feeding diets low in N for various periods of time and statistical methods of comparing growth curves may provide sufficient information for the design of growth trials that may be more conclusive.

The digestibility study provided evidence which suggests that BBGU may be superior to BBMGU as a N supplement and that the digestibilities of dry matter, energy and N in BBGU are as high as those of protein supplements such as soybean meal.

The experiment in which ruminal ammonia concentrations were measured after BBGU, BBMGU, soybean meal and urea were administered demonstrated that the administration of BBGU results in less rapid accumulation of ammonia than the administration of an amount of urea which was equivalent, in terms of N, to only half of the test dose of BBGU. The BBGU preparation used in the experiment contained 2.86% unreacted urea. The rapidity with which ammonia accumulates during the first 3 h after the administration of BBGU could likely be altered by changing the percentage of unreacted urea in the BBGU preparation. The manufacturing process apparently can be altered to allow for the inclusion of variable concentrations of unreacted urea. Perhaps higher concentrations of urea might be used in BBGU preparations used in diets high in digestible energy and low concentrations used in preparations used to supplement diets low in digestible energy, such as those consisting primarily of low-quality forages.

One of the potential dangers in the use of urea in animal diets is ammonia toxicity. BBGU appears to be essentially non-toxic even when fed as the majority of the diet.

BBGU appears to have potential as an NPN supplement for ruminants. Further testing is required to determine its effectiveness in supplying dietary N in various

types of ruminant diets. The potential of BBGU as a marketable product will depend on the costs of production, marketing and promotion, the availability and costs of other sources of dietary N and, of course, the efficacy of the product in ruminant diets.

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